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An Ultraviolet-Visible Spectrophotometer Automation System. Part II: User's Guide

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This document describes the UVVIS system from a user's perspective. It concentrates on how one interacts with the system, and on what system features one may use.

Two other related publications are "An Ultraviolet-Visible Spectrophotometer Automation System - Part I Functional Specifications," and "An Ultraviolet-Visible Spectrophotometer Automation System - Part III Program Documentation."

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AN ULTRAVIOLET-VISIBLE SPECTROPHOTOMETER AUTOMATION SYSTEM - PART 2 USER'S GUIDE

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FOREWORD

Environmental measurements are required to determine the quality of ambient waters and the character of waste effluents. The Environmental Monitoring and Support Laboratory - Cincinnati, conducts research to:

- Develop and evaluate techniques to measure the presence and concentration of physical, chemical, and radiological pollutants in water, wastewater, bottom sediments, and solid waste.
- Investigate methods for the concentration, recovery, and identification of viruses, bacteria, and other microbiological organisms in water, and to determine the responses of aquatic organisms to water quality.
- Develop and operate a computerized system for instrument automation leading to improved data collection, analysis, and quality control.

This report was developed in the Advanced Instrumentation Section of the Environmental Monitoring and Support Laboratory in the interest of advancing laboratory techniques and quality control through computerization.

Robert L. Booth

Robert L. Booth, Acting Director
Environmental Monitoring and Support
Laboratory - Cincinnati

ABSTRACT

The Ultraviolet-Visible Spectrophotometer (UVVIS) automation system facilitates computer-automated procedures for the analysis of chlorophyll concentrations and color evaluations of waters, as well as the quality assurance of the spectrophotometer. In addition to various types of reports, this system also offers plots of chlorophyll and color scan data for either plotting or non-plotting terminals. The UVVIS system also provides manual data-entry facilities for chlorophyll analysis and for three-filter photometer color analysis.

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SECTION 1

INTRODUCTION

The Ultraviolet-Visible Spectrophotometer (UVVIS) automation system is a set of nineteen cooperating programs. It helps you to test and maintain the quality assurance of the spectrophotometer. It also assists you in the analysis of chlorophyll concentrations and color evaluations (in ADMI color units) of waters. You may use the system to calculate chlorophyll concentrations by entering raw data absorbance or transmittance values. You may also employ it as a device for evaluating water colors by entering three-filter photometer data. The UVVIS system also provides you with: 1) the maintenance of spectrophotometric wavelength and transmittance calibrations per type of run; 2) replicate/duplicate chlorophyll concentration determinations; 3) the maintenance of spectrophotometer quality assurance statistics over time; 4) the maintenance of color standards calibration over time; and 5) various types of reports and raw data plots.

The system is designed to be self explanatory. Yet you should first become thoroughly acquainted with it through reading this document. Some of the more common terms used within this text are defined in the table found on the following page. To find out how to access the system, read the section about signing on to the system. To understand the way to answer the various prompts and questions given by the system, consult the section on General Principles of the System. To access the primary UVVIS functions, read the section on the Master Commands. Following that section, the automated and manual data-entry analysis procedures are described.

TABLE 1. COMMON TERMS USED IN THIS GUIDE

SESSION or RUN	- a computer-assisted process in which a complete set of samples is analyzed and evaluated; a RUN may be made in one of two modes: 1) automated, where the computer is used to acquire and reduce raw data from a computer-interfaced instrument; or 2) manual data-entry, where the computer is used to reduce user-supplied raw data.
ENTER	- the act in which a user supplies the computer with information by first typing letters/numbers on the terminal console and then pressing the carriage return (or "return") key.
DEFAULT	- some presently defined or pre-defined value or answer; its application is described in section 3.
DISPLAY	- the act in which a computer causes questions or information to be printed on the terminal console.
COMMAND	- a single-character or double-character mnemnonic entered by a user; it directs the computer to perform some necessary operation(s).
COMMAND-LINE	- a displayed query which is a line of single-character and/or double-character commands available to a user; explained in section 3.
OPTION	- a letter or number entered by a user; it directs the computer to perform some preferred task.
OPTION-SELECTION LINE	- a displayed query which is a list of lettered/numbered available choices; explained in section 3.
YES/NO QUESTION	- a displayed query which requires from the user an affirmative ("Yes") or negative ("No") entry; explained in section 3.
KEEP/CHANGE QUESTION	- a displayed query which requires alteration or acceptance by the user of some default value or definition; explained in section 3.

SECTION 2

SIGNING ON TO THE SYSTEM

The UVVIS system runs on a Nova 840 minicomputer (RDOS Rev.6.2, BASIC Rev.4.3) or an equivalent system. To sign on to the system you must use a terminal which is properly connected to the Nova and set for full duplex, no parity, on line, and 300 baud (or a higher baud rate, if permitted by the Nova system manager).

To get the attention of the Nova, press the ESCAPE key once. (Some Tektronix terminals do not have such a key. Instead, hold down the CONTROL and SHIFT keys, and press the K key.) If the Nova system is already servicing as many users as it can, then it will give you a corresponding message. But under normal circumstances, it will request your "account ID." The Nova system manager should have assigned you a pair of account ID's. (One is for the UVVIS system, while the other is simply for producing plots.) The account ID is not displayed on your terminal as you type.

Next, the Nova system may send some message of the day to your terminal. It then transfers you to the UVVIS system or the UVVIS plot function, depending on the account ID used. When the UVVIS system begins operation, you will face the available master commands, which are explained in section 4. The plot function behaves as described in the section on Plotting the Automated Chlorophyll/Color Analysis Raw Scan Data.

SECTION 3

GENERAL PRINCIPLES OF THE SYSTEM

There are several principles which apply to the UVVIS system in all circumstances. One important principle is that any time you answer a question, you must press the RETURN key.

To correct typing errors, press the RUBOUT key (or the DELETE key on some terminals). Each pressing of this key rubs out one character beginning with the last character you typed in. To delete an entire entry, press the backslash (\) key.

No commas are allowed in any alphabetic or numeric entry. Furthermore, if you supply an incorrect input, then the computer rings the bell in the terminal and repeats the question.

THE FOUR TYPES OF QUESTIONS WITH DEFAULT ANSWERS

A default answer is one which is supplied by the system or one which you have previously supplied. In all questions, there is one such default answer. The four types of questions are command lines, option-selection lines, yes/no questions, and keep/change questions.

A command line is a set of single and/or double character commands, with an "H" default command. For example,

```
MASTER COMMANDS:  C, L, Q, CM, LM, P, E, H(CR)  >>>
```

is the master command line. If you were to press only the RETURN key (CR for "carriage return"), then by default, it would be the same as entering the H command. (In any command line, the H command results in a terminal listing of explanations of the various commands.)

An option-selection line is a set of concisely stated options, each with its appropriate response in brackets. Some need a numerical input; for example,

```
REPORT OUTPUT:  TERMINAL[1], LINEPRINTER[2], CONTINUE 3(CR)   >>>
```

Others require an alphabetic response; for example,

```
DISPLAY INFO[I], PLOT[P] or END PLOTTING E(CR)   >>>
```

In the former example, if you were to press just the RETURN KEY, it would be the same as selecting option 3. In the latter example, a single RETURN would be equivalent to selecting the E option.

A yes/no question is a question which requires an affirmative or negative answer. An example is:

TEST CHANNEL OUTPUTS/FUNCTION ? N(CR) OR Y >>>

In this type of question, you may enter an N for 'no', or a Y for 'yes'. Simply pressing the RETURN KEY would be the same as an N response, the default answer.

A keep/change question is one which asks you to keep some default value/answer or to change it. An example is:

ANALYST'S NAME: JOHN Q. USER

KEEP(CR) OR CHANGE >>>

If you were to press the RETURN key, then the name currently displayed (that is, the default) would be accepted, and the system would proceed to the next step. However, were you to enter a new name, the same question would be repeated, accompanied by the correction.

SYSTEM COMMAND LEVELS AND THE ESCAPE KEY

The UVVIS system allows you to control the system functions by giving commands. There are several layers or "levels" of command in the system. The highest level is the master command level, which is shown above. If, for example, you were to command the automated chlorophyll analysis by entering a C, then after some preliminary operations, you would face the next lower level of command, which is:

CHLOROPHYLL ANALYSIS COMMANDS: T, M, R, A, D, E, H(CR) >>>

Here are presented commands which deal specifically with the automated chlorophyll analysis. If you were to command the analysis of a particular sample, you would enter an A. After taking care of a few more preparatory operations, you would arrive at the next lower command level, which is:

SAMPLE ANALYSIS COMMANDS: S, D, R, E, H(CR) >>>

Here are commands which pertain to one particular chlorophyll sample.

In every command line, the E and H commands work in the same way. The E command always invokes a termination of the present command level and a transfer to the next higher level. An E command chosen at the SAMPLE ANALYSIS COMMANDS line (above) would end the sample analysis and take you to the CHLOROPHYLL ANALYSIS COMMANDS line. And an E command chosen at the CHLOROPHYLL ANALYSIS COMMANDS line would end the chlorophyll analysis session and take you to the MASTER COMMANDS line.

The H command, which is always the default, causes a terminal display of single-line command descriptions. For example, at master command level, the output of the H command is:

```
C  ---  CHLOROPHYLL ANALYSIS (AUTOMATED)
L  ---  COLOR ANALYSIS (AUTOMATED)
Q  ---  QUALITY ASSURANCE FILTER TEST OF SPECTROPHOTOMETER
CM ---  CHLOROPHYLL ANALYSIS (MANUAL DATA-ENTRY)
LM ---  COLOR ANALYSIS (MANUAL DATA-ENTRY)
P  ---  PLOTS OF AUTOMATED ANALYSIS SCANS
E  ---  END OF UVVIS SESSION
H  ---  HELP
```

The ESCAPE key is important in relationship to command levels. If you are in a particular sequence of operations, and press the ESCAPE key, then the system will take you back to the command level at which you commanded that particular operation. (The only exception to this rule is escaping during an automated scan operation.)

Suppose, for example, that in the SAMPLE ANALYSIS COMMANDS line (above) you had pressed the S key, and had begun to process a sample replicate. By pressing the ESCAPE key during that sample processing operation, you would take yourself back to the SAMPLE ANALYSIS COMMANDS Line. It would be wise for you to make a test run of the UVVIS system in which you would try pressing the ESCAPE key at various places.

SECTION 4

THE MASTER COMMANDS

The master commands enable you to choose major functions of the UVVIS system. The master command line will be displayed on your terminal in the following form:

```
MASTER COMMANDS:  C, L, Q, CM, LM, P, E, H(CR)  >>>
```

Figure 1 shows the basic flow of operation in the UVVIS system, from the perspective of the master command level.

The C, L, and Q commands initiate automated runs of chlorophyll, color, and spectrophotometer quality assurance, respectively. The commands CM and LM are for manual data-entry runs of chlorophyll and color. The P command is for plotting raw scan data from automated runs of chlorophyll and color. An E command, as in all command lines, ends the present operation. At master command level, and E logs you off from the computer. The H command, as in every command line, creates a terminal listing of brief explanations of the commands in that particular line.

Since the master command level is the highest level, you cannot escape from it. Pressing the ESCAPE key will result only in a repetition of the master command line.

AUTOMATED RUNS -- COMMANDS C, L, AND Q

When you enter any one of the three automated run commands, then you must take care of several preparatory operations. The first time you command an automated run while using the UVVIS system, you must complete three minor tasks. The first two are keep/change questions in which you declare your name and the type of instrument. The third task is optional: a brief listing of instrument set-up instructions.

The first and all following automated run commands you type will be accompanied by two yes/no questions. These questions permit: 1) testing of channel outputs/function; and 2) checking/setting of the wavelength calibration equation.

If you elect to test the channels, then you will be given four options, presented as:

```
CHANNEL CHECKS:  TRANSMITTANCE[T], WAVELENGTH[W],
                 SCANNING[S], OR END CHECKING E(CR)   >>>
```

In the T and W options, the computer takes signals (A/D or "Analog-to-Digital" counts) from the appropriate data channel, and displays them to your terminal until you press the ESCAPE key, returning you to the channel check options. After pressing the S option, you will be instructed to set the spectrophotometer for scanning and to press the RETURN key. A scan will be made, and an appropriate evaluation message displayed. The E option (default) allows you to continue the sequence of preparatory operations.

Whether or not you choose to test the channels, the computer will make a few crucial examinations. It will first ensure that proper signals are coming from the wavelength and tramsittance channels. (Should there be any problem, you will be notified at your terminal, and the computer will go back to the master command line.) The computer will also search for valid wavelength calibration data. If the search proves negative, then you will have to decide between calibrating the wavelength or ending the session (in which case the system will log you off from the computer).

If you decide to check and/or set the wavelength calibration, you will be offered the following option-selection line:

WAVELENGTH CALIB.: CHECK(1), CALIBRATE(2), CONTINUE(3(CR)) >>>

The first two options are described in Appendix A. The last option lets you continue with the run.

Whether or not you deal with the wavelength calibration options, you will continue with the particulars of the chosen run. Consult the appropriate portions of this document for procedural details.

MANUAL DATA-ENTRY RUNS -- COMMANDS CM AND LM

Any time you type in either a CM or LM command, you will face two keep/change questions. One is for your name, and the other is for the instrument type. You will then proceed with the chosen run, as explained in the appropriate portion of this document.

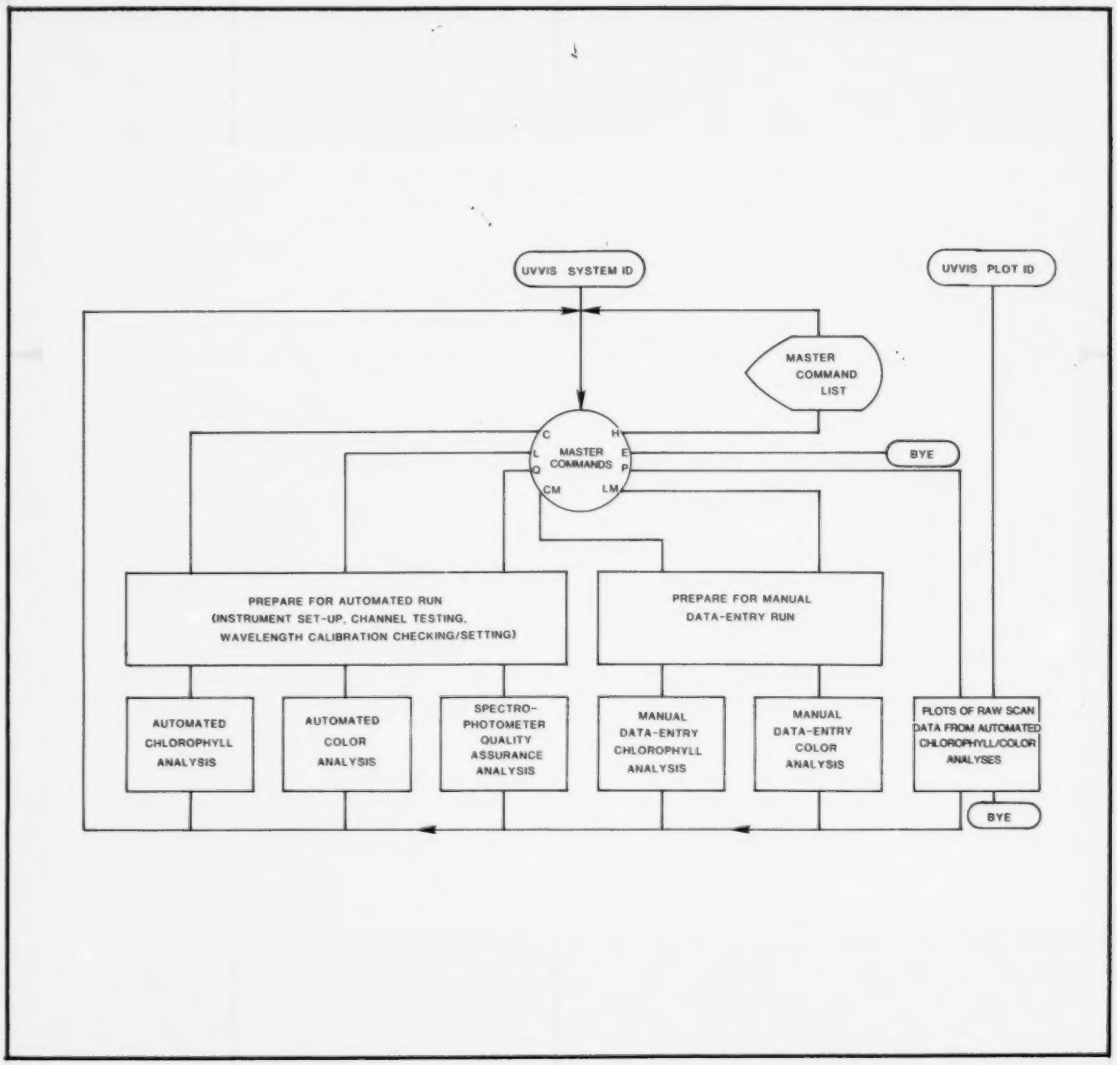


Figure 1. Flow of operation of the UVVIS system.

SECTION 5

ANALYZING SPECTROPHOTOMETER QUALITY ASSURANCE

The spectrophotometer quality assurance function of the UVVIS system aids you in the maintenance of the instrument's accuracy and precision. There are eight filters which you may use, each with certified data supplied by the National Bureau of Standards (NBS).[1] Five of the filters (2101 through 2105) are tristimulus filters, which possess characteristic tristimulus values X, Y and Z. The other three are linearity filters (1-232, 2-232 and 3-232), which exhibit particular transmittance/absorbance values at certain wavelengths. (Should your filters be of a different set with slightly different certified values, then have your system manager read Section 9 concerning changes to filter data.)

Filters are analyzed one at a time. For each filter you analyze, up to ten replicate scans may be made. You may analyze any filter more than once during the run. Figure 2 shows the flow of operation of the quality assurance analysis. The analysis commands and options are summarized in Table 2.

The accuracy of the spectrophotometer may be studied by comparing run results to the NBS certified values. The instrument's precision can be examined by comparing run results to the historical results. The historical results constitute the "standards" data. For each filter, as many as twenty-five sets or "replicates" of data may be maintained. As sets accumulate over time, the oldest data are replaced by the newest. When updating the standards with new results of a filter, all the replicates of that filter are added, each being considered as a set.

As an example, suppose that you have 20 sets of historical data for filter 2101. Suppose also that you run that same filter with six replicates. When you wish to update, all six replicates are added, making 26 sets of historical standards for filter 2101. However, since standards are limited to 25 sets per filter, the 26th set replaces the oldest (first) set of data.

THE QUALITY ASSURANCE COMMANDS

After pressing the Q command at master command level, and taking care of operations for the automated run, you will face the following command line:

QUALITY ASSURANCE COMMANDS: T, M, R, A, U, E, H(CR) >>>

The default H command will supply you with command letter explanations.

The T command enables you to check the present transmittance calibration and/or establish a fresh calibration throughout the scan range (400 to 700 nanometers). The most common reference medium used in filter analysis is air. The procedures for checking and/or setting the transmittance calibration are described in Appendix B. You must set the speed of the scanning mechanism before the first transmittance calibration is ever done. Should it not be defined, then an appropriate message is displayed at your terminal, and the T command not processed.

The M command is for checking and/or setting the scanning mechanism speed. Any time you wish to change speeds, you must use this command. Consult Appendix C for details on this operation.

An R command tells the system to prepare a summary report for the quality assurance run. Basic information on each filter is prepared, as shown in Figure 5. You may request that the report be sent to your terminal or to the lineprinter. (See Appendix D for some brief advice on obtaining reports.) Keep in mind that all precision and accuracy evaluations reflect the current standards statistics, even those evaluations for filter data which have already been incorporated into the present statistics.

Use the A command to analyze a filter. You will be asked to enter a filter number with the question:

SELECT FILTER [1 TO 8] or H(CR) >>>

By typing an H, or just pressing the RETURN key, you will get a list of the numbers and their corresponding designations such as 2101 or 2102, and so forth. Once you select the filter, you will proceed with the filter analysis as described below in the filter analysis commands.

With the U command you may update the historical standards statistics. A list of options will first be displayed:

STANDARDS UPDATE: LIST FILTERS[1], UPDATE BY DESIGNATION[2],
UPDATE BY LIST NUMBER[3], CONTINUE[4(CR)] >>>

Option 1 provides you with a numbered list of the filters analyzed, showing each designation and how many replicates. In option 2, you enter the filter designation. An invalid designation will be refused. If you have analyzed the same filter more than once during the run, then the earliest non-updated one will be added. In option 3, you merely supply the list number from option 1. Option 4 returns you to the quality assurance commands, (as does a successful update option 2 or 3).

Regarding options 2 and 3, should the filter's data already have been added to the historical standards, then you will be notified, and the data will not be added. Note that since all replicates are added, you must take care of any relicate deletions under command D of the filter analysis procedure described below.

The E command consummates the quality assurance analysis session and sends you back to master command level. If, after choosing the E command, you absolutely must add more filter analyses to the ended run (without losing the past data), then have your system manager consult section 9 of this document.

The Filter Analysis Commands

Having just entered the A quality assurance command and selected a filter, you are given the following command line:

FILTER ANALYSIS COMMANDS: S, D, R, E, H(CR) >>>

The H default command lists command explanations. The E command informs the system that you have finished studying the present filter, and want to return to the quality assurance comands.

Use the S command to scan and compute results for one replicate of the filter. You will be told to set the wavelength dial at 705 nanometers, to set the instrument to scan, and to press the RETURN key. Having done this, you must wait while the scan and data acquisition takes place. (If you ESCAPE while the instrument is scanning, you will be questioned whether or not to restart the scan. If you choose not to, you will again face the filter analysis commands, and no data from the replicate will have been stored.) After the scan, the results will be concisely displayed, and you'll be asked:

REJECT RESULTS ? N(CR) OR Y >>>

Type an N (or just RETURN) to save the replicate's data. Type a Y to ignore its results. You may also delete replicate data later by using the D command.

The D command allows you to delete the data of a replicate. You will be asked to enter the number of the replicate. After the deletion, a message will be sent to the terminal. The remaining replicates will be renumbered in consecutive order, beginning with the number one. Note that it is convenient to first look at a filter report to decide if a replicate need be deleted.

An R command tells the system to prepare a report which summarizes replicate results for the present filter. (See Appendix D on Obtaining Reports.) Two example reports, one for a linearity filter and the other for a tristimulus filter, are shown in Figures 3 and 4.

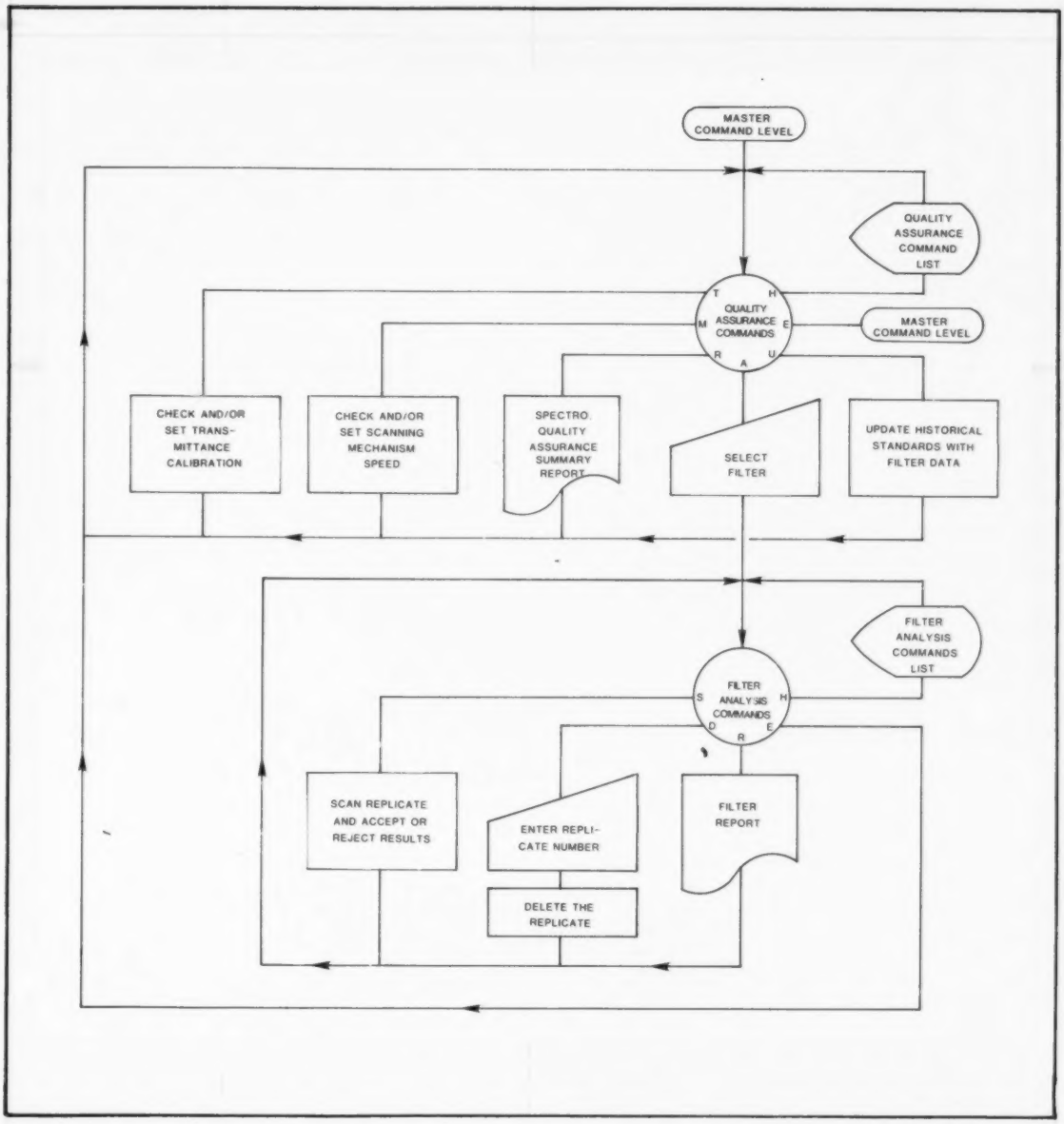


Figure 2. Flow of operation of the UVVIS spectrophotometer quality assurance analysis.

TABLE 2. SPECTROPHOTOMETER QUALITY ASSURANCE (SQA) ANALYSIS SUMMARY

SQA ANALYSIS COMMANDS:

T -- TRANSMITTANCE CALIBRATION CHECK/SET:
+ TASK: ADJUSTMENT OF SPECTROPHOTOMETER AT 0% AND 100% TRANSMITTANCE LEVELS;
+ OPTIONS: 1. CHECK OF CALIBRATION;
2. CALIBRATION AT 0% AND 100% TRANSMITTANCE;
3. CONTINUATION OF ANALYSIS SESSION (DEFAULT OPTION).

M -- SCANNING MECHANISM SPEED CHECK/SET:
OPTIONS: 1. CHECK OF PRESENT SPEED;
2. SETTING OF SPEED;
3. CONTINUATION OF ANALYSIS SESSION (DEFAULT OPTION).

R -- SUMMARY REPORT OF SQA ANALYSIS SESSION:
OPTIONS: 1. TERMINAL DISPLAY OF REPORT;
2. LINEPRINTER COPY OF REPORT;
3. CONTINUATION OF ANALYSIS SESSION (DEFAULT OPTION).

A -- ANALYSIS OF ONE FILTER:
+ TASK: SELECTION OF FILTER TO BE ANALYZED;
+ FILTER ANALYSIS COMMANDS:

S -- SCAN OF ONE FILTER REPLICATE:
+ TASK: PLACEMENT OF FILTER IN SPECTROPHOTOMETER CHAMBER;
+ TASK: SETTING OF WAVELENGTH DIAL AND INITIATION OF SCAN BY PRESSING RETURN KEY;
+ INFORMATION: TERMINAL DISPLAY OF SCAN RESULTS;
+ DECISION: ACCEPTANCE (DEFAULT ANSWER) OR REJECTION OF RESULTS.

D -- DELETION OF DATA OF ONE FILTER REPLICATE:
+ TASK: ENTRY OF REPLICATE NUMBER;
+ INFORMATION: TERMINAL DISPLAYED MESSAGE OF DELETION.

R -- INTERMEDIATE QUALITY ASSURANCE REPORT:
OPTIONS: 1. TERMINAL DISPLAY OF REPORT;
2. LINEPRINTER COPY OF REPORT;
3. CONTINUATION OF FILTER ANALYSIS (DEFAULT OPTION).

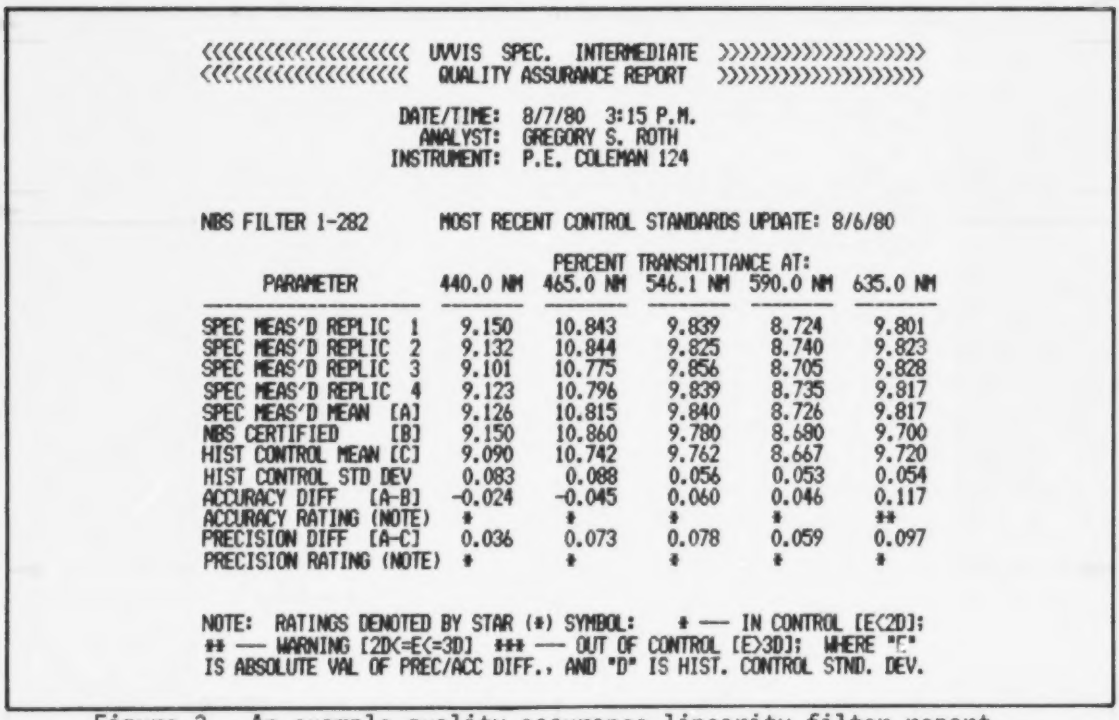
E -- END OF ANALYSIS OF THIS FILTER; RETURN TO SQA ANALYSIS COMMAND LEVEL.

H -- HELP: TERMINAL DISPLAY OF FILTER ANALYSIS COMMAND EXPLANATIONS (DEFAULT COMMAND).

U -- UPDATE OF HISTORICAL STANDARDS WITH ANALYSIS DATA:
OPTIONS: 1. TERMINAL DISPLAYED LISTING OF FILTERS ANALYZED DURING SESSION;
2. UPDATE BY FILTER DESIGNATION:
+ TASK: ENTRY OF FILTER DESIGNATION;
+ INFORMATION: TERMINAL DISPLAYED MESSAGE OF UPDATE;
+ CONTINUATION OF ANALYSIS SESSION.
3. UPDATE BY FILTER NUMBER (FROM OPTION 1 LIST):
+ TASK: ENTRY OF OPTION 1 LIST NUMBER;
+ INFORMATION: TERMINAL DISPLAYED MESSAGE OF UPDATE;
+ CONTINUATION OF ANALYSIS SESSION.
4. CONTINUATION OF ANALYSIS SESSION (DEFAULT OPTION).

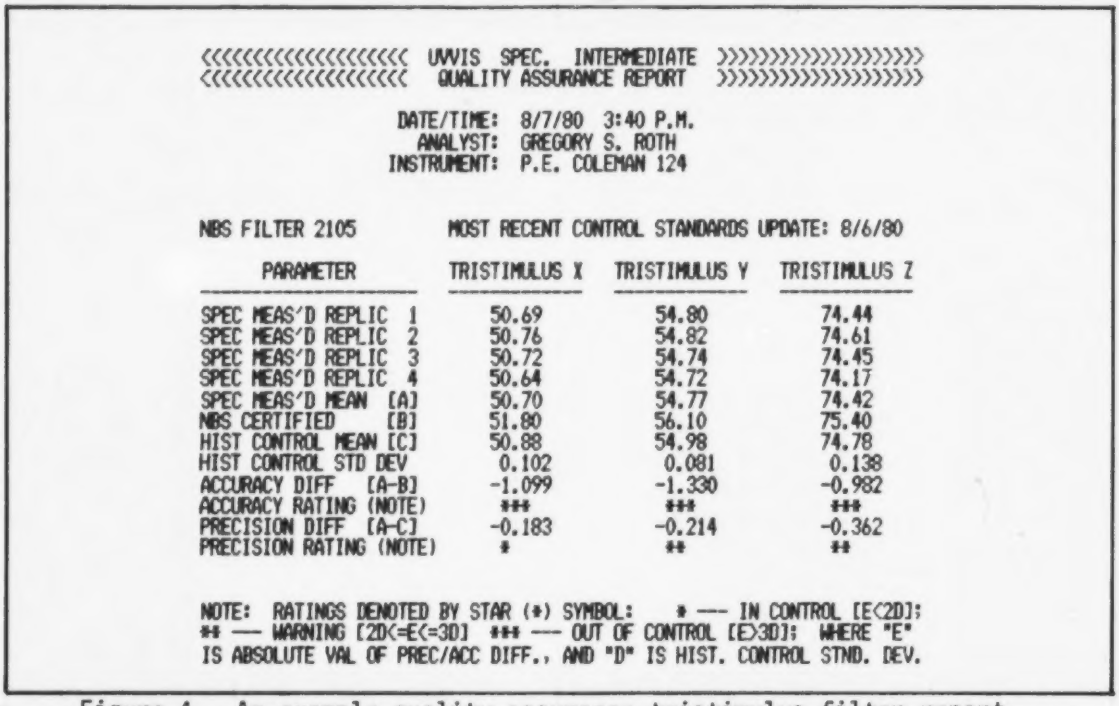
E -- END OF SQA ANALYSIS SESSION; RETURN TO UVVIS MASTER COMMAND LEVEL.

H -- HELP: TERMINAL DISPLAY OF SQA ANALYSIS COMMAND EXPLANATIONS (DEFAULT COMMAND).



```
<<<<<<<<<<<<<<<<<<<<  UVVIS SPEC.  INTERMEDIATE  >>>>>>>>>>>>>>>>>>>>
<<<<<<<<<<<<<<<<<<<<  QUALITY ASSURANCE REPORT  >>>>>>>>>>>>>>>>>>>>

                    DATE/TIME:  8/7/80  3:15 P.M.
                      ANALYST:  GREGORY S. ROTH
                   INSTRUMENT:  P.E. COLEMAN 124

NBS FILTER 1-282        MOST RECENT CONTROL STANDARDS UPDATE: 8/6/80

                                PERCENT TRANSMITTANCE AT:
      PARAMETER          440.0 NM  465.0 NM  546.1 NM  590.0 NM  635.0 NM
-------------------------------------------------------------------------
SPEC MEAS'D REPLIC  1      9.150     10.843     9.839     8.724     9.801
SPEC MEAS'D REPLIC  2      9.132     10.844     9.825     8.740     9.823
SPEC MEAS'D REPLIC  3      9.101     10.775     9.856     8.705     9.828
SPEC MEAS'D REPLIC  4      9.123     10.796     9.839     8.735     9.817
SPEC MEAS'D MEAN   [A]     9.126     10.815     9.840     8.726     9.817
NBS CERTIFIED      [B]     9.150     10.860     9.780     8.680     9.700
HIST CONTROL MEAN [C]      9.090     10.742     9.762     8.667     9.720
HIST CONTROL STD DEV       0.083      0.088     0.056     0.053     0.054
ACCURACY DIFF    [A-B]    -0.024     -0.045     0.060     0.046     0.117
ACCURACY RATING (NOTE)         *          *         *         *        **
PRECISION DIFF   [A-C]     0.036      0.073     0.078     0.059     0.097
PRECISION RATING (NOTE)        *          *         *         *         *

NOTE:  RATINGS DENOTED BY STAR (*) SYMBOL:    * --- IN CONTROL [E<2D];
** --- WARNING [2D<=E<=3D]  *** --- OUT OF CONTROL [E>3D];  WHERE "E"
IS ABSOLUTE VAL OF PREC/ACC DIFF., AND "D" IS HIST. CONTROL STND. DEV.
```

Figure 3. An example quality assurance linearity filter report.



```
<<<<<<<<<<<<<<<<<<<<  UVVIS SPEC.  INTERMEDIATE  >>>>>>>>>>>>>>>>>>>>
<<<<<<<<<<<<<<<<<<<<  QUALITY ASSURANCE REPORT  >>>>>>>>>>>>>>>>>>>>

                    DATE/TIME:  8/7/80  3:40 P.M.
                      ANALYST:  GREGORY S. ROTH
                   INSTRUMENT:  P.E. COLEMAN 124

NBS FILTER 2105         MOST RECENT CONTROL STANDARDS UPDATE: 8/6/80

      PARAMETER          TRISTIMULUS X   TRISTIMULUS Y   TRISTIMULUS Z
-------------------------------------------------------------------------
SPEC MEAS'D REPLIC  1          50.69           54.80           74.44
SPEC MEAS'D REPLIC  2          50.76           54.82           74.61
SPEC MEAS'D REPLIC  3          50.72           54.74           74.45
SPEC MEAS'D REPLIC  4          50.64           54.72           74.17
SPEC MEAS'D MEAN   [A]          50.70           54.77           74.42
NBS CERTIFIED      [B]          51.80           56.10           75.40
HIST CONTROL MEAN [C]          50.88           54.98           74.78
HIST CONTROL STD DEV            0.102           0.081           0.138
ACCURACY DIFF    [A-B]         -1.099          -1.330          -0.982
ACCURACY RATING (NOTE)            ***             ***             ***
PRECISION DIFF   [A-C]         -0.183          -0.214          -0.362
PRECISION RATING (NOTE)            *              **              **

NOTE:  RATINGS DENOTED BY STAR (*) SYMBOL:    * --- IN CONTROL [E<2D];
** --- WARNING [2D<=E<=3D]  *** --- OUT OF CONTROL [E>3D];  WHERE "E"
IS ABSOLUTE VAL OF PREC/ACC DIFF., AND "D" IS HIST. CONTROL STND. DEV.
```

Figure 4. An example quality assurance tristimulus filter report.

****

UVVIS

SPECTROPHOTOMETER QUALITY ASSURANCE SUMMARY

****

DATE/TIME: 8/7/80 3:48 P.M.
ANALYST: GREGORY S. ROTH
INSTRUMENT: P.E. COLEMAN 124

NBS FILTER 1-282 MOST RECENT CONTROL STANDARDS UPDATE: 8/6/80

PARAMETER	PERCENT TRANSMITTANCE AT: 440.0 NM	465.0 NM	546.1 NM	590.0 NM	635.0 NM
SPEC MEAS'D MEAN [A]	9.126	10.815	9.840	8.726	9.817
NBS CERTIFIED [B]	9.150	10.860	9.780	8.680	9.700
HIST CONTROL MEAN [C]	9.099	10.760	9.781	8.681	9.745
HIST CONTROL STD DEV	0.073	0.083	0.059	0.053	0.063
ACCURACY DIFF [A-B]	-0.024	-0.045	0.060	0.046	0.117
ACCURACY RATING (NOTE)	*	*	*	*	*
PRECISION DIFF [A-C]	0.027	0.055	0.058	0.044	0.073
PRECISION RATING (NOTE)	*	*	*	*	*

NBS FILTER 2102 MOST RECENT CONTROL STANDARDS UPDATE: 8/6/80

PARAMETER	TRISTIMULUS X	TRISTIMULUS Y	TRISTIMULUS Z
SPEC MEAS'D MEAN [A]	51.78	49.37	6.04
NBS CERTIFIED [B]	51.50	48.90	5.60
HIST CONTROL MEAN [C]	51.68	49.26	6.02
HIST CONTROL STD DEV	0.177	0.226	0.076
ACCURACY DIFF [A-B]	0.282	0.467	0.438
ACCURACY RATING (NOTE)	*	**	***
PRECISION DIFF [A-C]	0.106	0.103	0.022
PRECISION RATING (NOTE)	*	*	*

NBS FILTER 2105 MOST RECENT CONTROL STANDARDS UPDATE: 8/6/80

PARAMETER	TRISTIMULUS X	TRISTIMULUS Y	TRISTIMULUS Z
SPEC MEAS'D MEAN [A]	50.70	54.77	74.42
NBS CERTIFIED [B]	51.80	56.10	75.40
HIST CONTROL MEAN [C]	50.80	54.89	74.62
HIST CONTROL STD DEV	0.124	0.129	0.241
ACCURACY DIFF [A-B]	-1.099	-1.330	-0.982
ACCURACY RATING (NOTE)	***	***	***
PRECISION DIFF [A-C]	-0.101	-0.119	-0.201
PRECISION RATING (NOTE)	*	*	*

NOTE: RATINGS DENOTED BY STAR (*) SYMBOL: * — IN CONTROL [E<2D]; ** — WARNING [2D<=E<=3D] *** — OUT OF CONTROL [E>3D]; WHERE "E" IS ABSOLUTE VAL OF PREC/ACC DIFF., AND "D" IS HIST. CONTROL STND. DEV.

Figure 5. An example summary report for a spectrophotometer quality assurance run.

SECTION 6

ANALYZING CHLOROPHYLL

The UVVIS chlorophyll procedure, both in the automated and manual data-entry modes, employs the trichromatic method.[2] For this method, there are three available versions of trichromatic equations. In an automated run, you may direct the system to search for the peak absorbance rather than the abosrbance at the method's "peak absorbance" wavelength.

The UVVIS system procedure facilitates two formats for the measurement of chlorophyll samples. In one format, you measure different extracts of corresponding grab samples from the same source. In the other format, you measure replicates from the same extract of a sample. Consult Figures 6 and 7 for pictorial representations of the two formats.

In both the automated and manual procedures, the system offers detailed reports for each sample, and a summary report for the run. Observe the example reports in Figures 8 and 10. Note that these reports exhibit a run done under format 2, as described above. In an automated run, the system also prepares plots of raw scan data from each replicate, such as the plot in Figure 9.

The analysis of chlorophyll requires the definition of several factors or "parameters." Some parameters apply to the entire run, that is, to every replicate of every sample in the run. Others apply only to a sample (and all of its replicates). Some apply to each replicate. Table 3 summarizes the scope of the chlorophyll parameters, and also describes their form such as units of measurement and available options.

THE AUTOMATED RUN

The automated run begins by asking you if you wish to check and/or change the default parameters. (However, for the very first chlorophyll run of the system, the system assumes a "yes" response.) Should you decide to examine the parameters, then the system will proceed to offer a series of keep/change questions for each parameter which applies to the run. Figure 11 simulates an example run parameters examination. Consult Table 3 for explanations of the run-scope parameters. The system then offers you the chlorophyll analysis commands. Figure 12 shows the flow of operation of the UVVIS chlorophyll analysis. The commands and options of the automated and manual data-entry analyses are summarized in Tables 4 and 5.

The Chlorophyll Analysis Commands

After selecting the C command at master command level and preparing the automated run, you will be given the following set of commands:

CHLOROPHYLL ANALYSIS COMMANDS: T, M, R, A, D, E, H(CR) >>>

The default H command yields a list of command letter explanations.

The T command enables you to check the present transmittance calibration and/or establish a fresh calibration throughout the scan range (420 to 760 nanometers). The reference medium for chlorophyll analysis is 90% aqueous acetone solution. The procedures for checking and/or setting the transmittance calibration are described in Appendix B. You must set the speed of the scanning mechanism before the first transmittance calibration is ever done. Should it not be defined, then an appropriate message is displayed at your terminal, and the T command is not processed.

The M command is for checking and/or setting the scanning mechanism speed. Any time you wish to change speeds, you must use this command. Consult Appendix C for details on this operation.

An R command requests that the system prepare a report which summarizes the general results for each sample. Before it is prepared, you will be asked whether or not you want raw data-- absorbance values-- in the report. An example of a report with raw data is shown in Figure 10. Only means of replicates are output. If you want information for each replicate, you must get the more detailed intermediate report which is available in the sample analysis commands. Biomass data are in the report, provided you have entered those data. See the brief Appendix D concerning terminal and/or lineprinter reports.

The A command permits you to analyze a sample. You must first specify an eight-character identifier and the dilution factor for the sample.

When defining the sample identifier, you will be asked:

SAMPLE ID: [USER-SET OR AUTOMATIC(CR)] >>>

At this point, you may enter an identifier, or just press the RETURN key and let the system automatically assign it. A system-defined ID will be of the form "Smmddnnn," where mm is the month date, dd is the day date, and nnn counts the samples. For example, the second sample of a run made on July 23rd would be identified as "S0723002." When you define the ID yourself, as many as eight characters may be typed. If less than eight characters, the ID will be padded on the left with zeroes. Any blanks will be changed to zeroes. For example, if you were to type in "CHL 3," then the ID would be "000CHL03."

After assigning the sample ID, you must define the dilution factor for the sample in a keep/change question. For the first sample, you must enter the value. Thereafter, merely keep the current (default) value by pressing

the RETURN key, or enter a new value, which will become the new default answer. Then you proceed to analyze the sample, using the sample analysis commands explained below.

Under each D command, you may delete one sample from the run. The computer will offer the options:

```
SAMPLE DELETION:  LIST SAMPLES[1], DELETE BY ID[2],
                  DELETE BY LIST NUMBER [3], CONTINUE[4(CR)]  >>>
```

Option 1 lists the IDs of the undeleted samples, numbering them in consecutive order. In option 2, you enter the sample ID, while in option 3, the list number from option 1. Option 4 gives you the chlorophyll analysis commands again. (A successful completion of the deletion option 2 or 3 will also take you back to the chlorphyll analysis command line.)

In an automated run, a successful sample deletion affects the raw data plots. Plots for each replicate of the deleted sample are marked as "DELETED." In this way, you may still observe those plots, and they will be noted as having been deleted.

An E command informs the system that you have finished studying all the samples. The system then rises to master command level and offers you the master commands. If, after choosing the E command, you wish to add sample analyses to the ended run without losing past data, then have your system manager consult Section 9 of this document.

The Filter Analysis Commands --

Once the identifier and dilution factor for the samples have been established, you will be offered these commands:

```
SAMPLE ANALYSIS COMMANDS:  S, D, R, E, H(CR)  >>>
```

The default H command briefly describes the other sample analysis commands at your terminal.

With the S command, you scan one replicate. You will first be asked to define one or two parameters: 1) the sample volume/area; and 2) the biomass concentration, only if you are measuring it. The units in which these parameters are to be defined are shown in Table 3. The values are assigned by keep/change questions.

The unacidified replicate is then scanned. The system will direct you to set the spectrophotometer for scanning at 765 nanometers, and to press the RETURN key. This latter operation initiates the scan and data collection. After the scan, results will be displayed for the unacidified replicate. You may reject or accept the results via a yes/no question. Should you reject them, the computer will again tell you to set-up for the unacidified scan. Should you accept them, if you have specified (at the beginning of the run) that only unacidified samples are to be studied, then

the replicate analysis will be considered complete, and you will be offered the sample analysis commands.

Otherwise, the computer will ask you to prepare for the acidified scan. Be careful to coordinate the timing of the acidification process with the scan preparation. By pressing the RETURN key, you initiate the second scan of the replicate. After the data collection, acidified scan results will be displayed at your terminal. You may accept or reject these results. Rejection provokes repeated scan preparation instructions, while acceptance leads to the sample analysis commands.

The D command permits you to delete the data of one replicate of the present sample. The system will ask:

SAMPLE XXXXXXXX REPLICATE TO BE DELETED [1 TO N] >>>

where XXXXXXXX is the sample ID, and N is the number of undeleted replicates (from 1 to 5). Type in the replicate number. That replicate will be deleted, and the remaining ones renumbered consecutively, beginning with the number one. In automated runs, the raw data for the plot of that replicate is marked as "REPLICATE X," and the others are renumbered. Thus, you may still see the plot of the bad replicate.

The R command calls upon the system to prepare a report which details raw absorbance data and concentration results for each replicate of the run, as exemplified in Figure 8. (See Appendix D on obtaining terminal and/or lineprinter reports.)

With an E command, you tell the system that you are finished with a particular sample. The sample commands are again given to you.

THE MANUAL DATA-ENTRY RUN

The manual data-entry run begins by asking if you wish to check and/or change the default parameters. (However, for the very first chlorophyll run of the system, the system assumes a "yes" response.) Should you decide to examine the parameters, then the system will proceed to offer a series of keep/change questions for each parameter which applies to the run. Figure 11 simulates an example run parameters examination. Consult Table 3 for explanations of the run-scope parameters. The system then offers you the chlorophyll analysis commands. Figure 12 shows the flow of operation of the UVVIS chlorophyll analysis.

The Chlorophyll Analysis Commands

After selecting the CM master command and preparing for the manual data-entry run, the system offers you the following commands:

CHLOROPHYLL ANALYSIS COMMANDS: R, A, D, E, H(CR) >>>

The default H command produces a list of command letter explanations. The remaining four commands (R, A, D, and E) operate just as in the automated

run. When you command the analysis of a particular sample (by entering A), and take care of defining the sample ID and dilution factor (as explained for the automated run), you will be offered the manual data-entry sample analysis commands.

The Sample Analysis Commands --

The sample analysis command line is:

SAMPLE ANALYSIS COMMANDS: I, D, R, E, H(CR) >>>

The H command explains the other commands.

With an I command,you supply as input the data of one replicate. The computer will request your raw data readings at the specific wavelengths of the trichromtic method. You may substitute found peak absorbance values for method peaks, and as a matter of formality, have them so indicated on reports by selecting the "found" Peak Absorbance run parameter. The computer will ask for data from the unacidified replicate, and also from the acidified replicate if you have so directed at the run's start. You supply the raw data in percent transmittance values or absorbance units, depending on which Data Entry run parameter option you chose. Results for the unacidified (and if measured, acidifed) replicate are then computed and briefly displayed at your terminal. If you reject the results through the yes/no question "REJECT RESULTS ? N(CR) OR Y ," then the system will again request the raw data. Otherwise, the system will again request the entry of the raw data.

The remaining three commands (D, R, and E) are described in the Sample Analysis Commands material for an automated run.

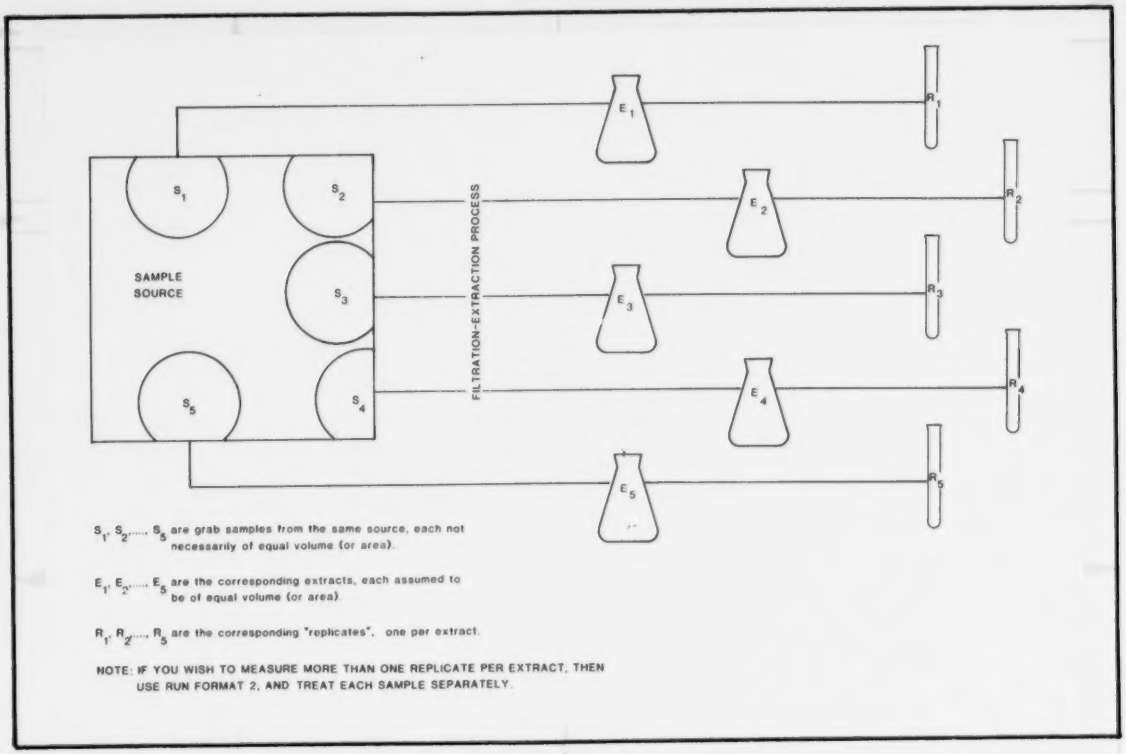

SAMPLE SOURCE
S1
S2
S3
S4
S5
FILTRATION-EXTRACTION PROCESS
E1
E2
E3
E4
E5
R1
R2
R3
R4
R5
S1, S2,..... S5 are grab samples from the same source, each not necessarily of equal volume (or area).
E1, E2,..... E5 are the corresponding extracts, each assumed to be of equal volume (or area).
R1, R2,..... R5 are the corresponding "replicates", one per extract.
NOTE: IF YOU WISH TO MEASURE MORE THAN ONE REPLICATE PER EXTRACT, THEN USE RUN FORMAT 2, AND TREAT EACH SAMPLE SEPARATELY.

Figure 6. Run format 1 for the UVVIS chlorophyll analysis.

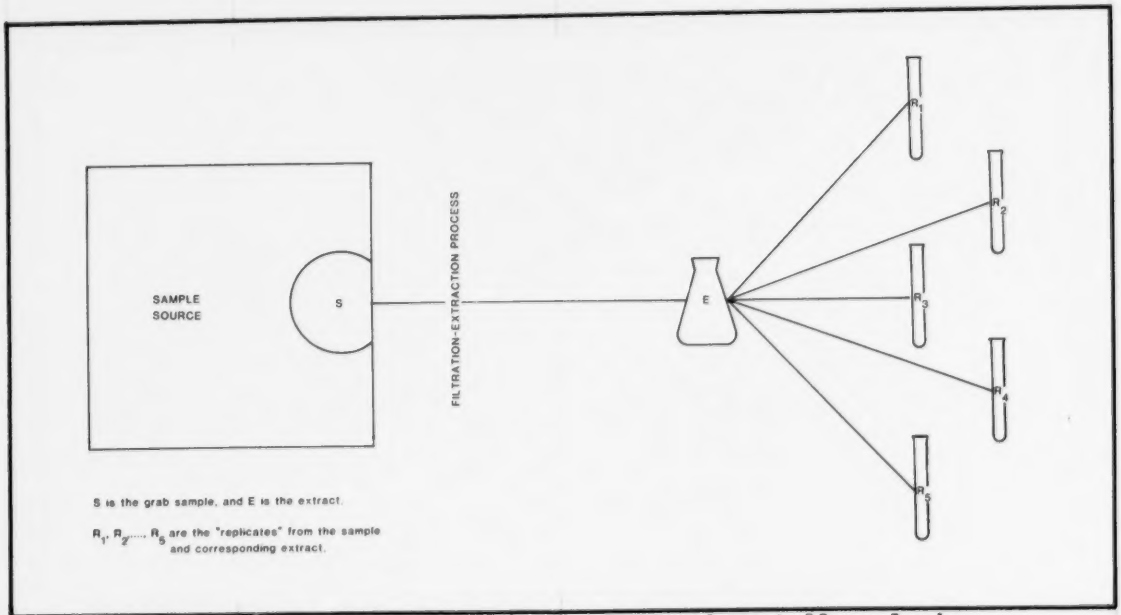


Figure 7. Run format 2 for the UVVIS chlorophyll analysis.

<<<<<<<<<< UVVIS INTERMEDIATE CHLOROPHYLL COMPUTATION >>>>>>>>>>

(DATE/TIME: 8/7/80 4:15 P.M.)

ANALYST: GREGORY S. ROTH
STATION/STUDY: UVVIS TEST SAMPLES
EXTRACTION METHOD: GRIND
COMPUTATION METHOD: UNESCO
INSTRUMENT: P.E. COLEMAN 124

SAMPLE ID: S0807001
SAMPLE TYPE: PLANKTON
EXTRACT VOLUME: 1.00 ML
CELL PATH LENGTH: 1.00 CM
DILUTION FACTOR: 1.000

SAMPLE RAW DATA	VOLUME [LITERS]	(BEFORE ACIDIFICATION) ABSORB. AT 630 NM	645 NM	PEAK	750 NM	(AFTER) ABS. AT PEAK	750 NM
REPL. 1	1.00E-03	0.194	0.325	0.729	0.007	0.594	0.063
REPL. 2	1.00E-03	0.200	0.327	0.747	0.011	0.612	0.064
REPL. 3	1.00E-03	0.198	0.312	0.744	0.010	0.612	0.065
REPL. 4	1.00E-03	0.204	0.320	0.752	0.014	0.617	0.072
MEAN	1.00E-03	0.199	0.321	0.743	0.010	0.609	0.066
ST. DV.	1.075E-09	0.0041	0.0065	0.0100	0.0030	0.0104	0.0040
%REL SD	0.000	2.047	2.027	1.343	28.342	1.713	6.028

SAMPLE UNCORR.	CHL-A [MG/M^3]	CHL-B [MG/M^3]	CHL-C [MG/M^3]	TOTAL CHL [MG/M^3]	RATIO CHL-A/B	RATIO CHL-A/C
REPL. 1	7737.215	3140.897	1442.742	12320.848	2.46	5.36
REPL. 2	7907.000	3034.513	1480.489	12422.000	2.61	5.34
REPL. 3	7907.391	2769.604	1683.816	12360.805	2.86	4.70
REPL. 4	7951.227	2810.223	1686.181	12447.629	2.83	4.72
MEAN	7875.707	2938.809	1573.306	12387.820	2.69	5.03
ST. DV.	94.6333	178.1045	129.8915	57.6209	0.187	0.373
%REL SD	1.20	6.06	8.26	0.47	6.96	7.42

SAMPLE CORR.	UNCOR/COR ABS RATIO	PHEO-A [MG/M^3]	CHL-A [MG/M^3]	TOTAL CHL [MG/M^3]	RATIO CHL-A/B	RATIO CHL-A/C
REPL. 1	1.361	4814.609	5116.031	9699.664	1.63	3.55
REPL. 2	1.343	5235.711	5024.352	9539.352	1.66	3.39
REPL. 3	1.342	5240.805	4994.988	9448.402	1.80	2.97
REPL. 4	1.353	5056.117	5151.027	9647.430	1.83	3.05
MEAN	1.350	5086.809	5071.598	9583.711	1.73	3.24
ST. DV.	0.0090	200.7660	73.9022	112.2220	0.103	0.275
%REL SD	0.67	3.95	1.46	1.17	5.95	8.48

Figure 8. An example report for one chlorophyll sample.

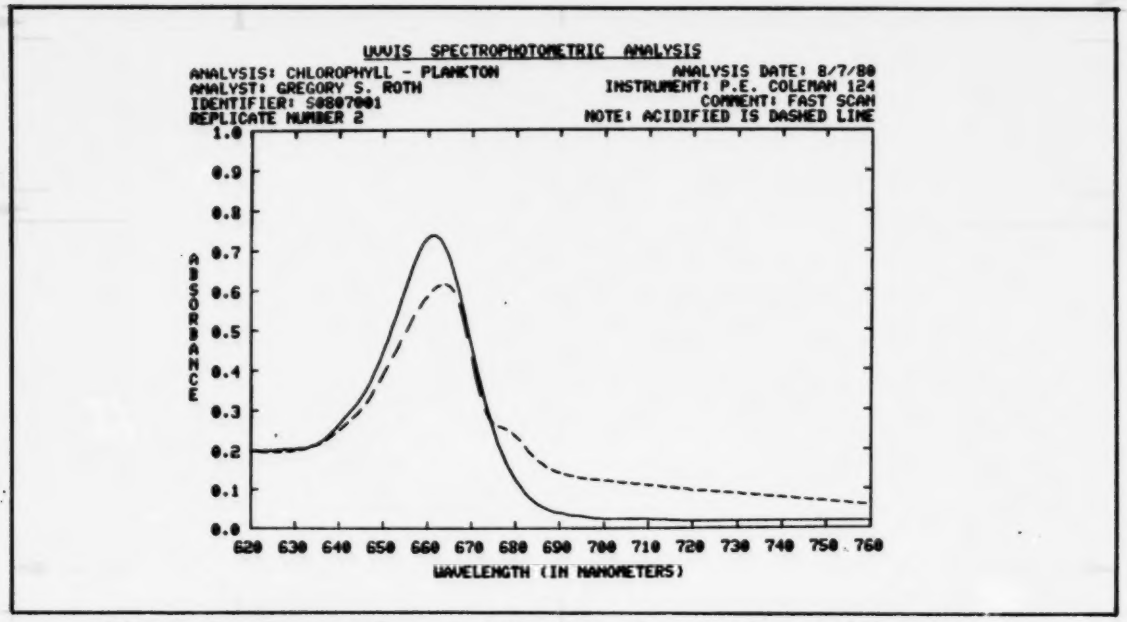


Figure 9. An example raw data plot of one chlorophyll replicate.

UVVIS

CHLOROPHYLL COMPUTATION SUMMARY

DATE/TIME: 8/7/80 5:28 P.M.

ANALYST: GREGORY S. ROTH
STAION/STUDY: UVVIS TEST SAMPLES
EXTRACTION METHOD: GRIND
COMPUTATION METHOD: UNESCO
INSTRUMENT: P.E. COLEMAN 124

DATE ANALYZED: 8/ 7/80
SAMPLE TYPE: PLANKTON
EXTRACT VOLUME: 1.00 ML
CELL PATH LENGTH: 1.00 CM
COMMENT: FAST SCAN

[RAW DATA] SAMPLE ID NO.	SAMPLE VOLUME [LITERS]	(BEFORE ACIDIFICATION) ABSORB. AT 630 NM	645 NM	PEAK	750 NM	(AFTER) ABS. AT PEAK	750 NM
S0807001	1.00E-03	0.1990	0.3211	0.7431	0.0104	0.6089	0.0660
S0807002	1.00E-03	0.1978	0.3271	0.7379	0.0109	0.5981	0.0673
S0807003	1.00E-03	0.2046	0.3247	0.7445	0.0145	0.6101	0.0639

SAMPLE ID NO.	CHL-A (CORREC.) [MG/M^3]	PHEO-A [MG/M^3]	CHL-A (UNCORR.) [MG/M^3]	CHL-B [MG/M^3]	CHL-C [MG/M^3]	TOTAL CHLORO. [MG/M^3]
S0807001	5071.598	5086.809	7875.707	2938.809	1573.306	9583.711
S0807002	5245.164	4686.086	7798.070	3081.182	1432.650	9758.996
S0807003	4911.551	5309.203	7846.227	2931.514	1675.416	9518.477

Figure 10. An example summary report for a chlorophyll run.

TABLE 3. CHLOROPHYLL RUN PARAMETERS -- THEIR SCOPE AND FORM

PARAMETER	SCOPE	FORM (UNITS/OPTIONS)
Station/Study	RUN	up to 18 characters long
Sample Type	RUN	Plankton(P) or Periphyton (E)
Extract Volume	RUN	milliliters
Extraction Method	RUN	up to 18 characters long
Cell Path Length	RUN	centimeters
Computation Method	RUN	UNESCO(U), Strickland-Parsons(S), or Jeffrey-Humphrey(J)
Sample Acidification	RUN	SAMPLES Unacidified only(U) or unacidified and Acidified(A)
Peak Absorbance	RUN	Method(M) or Found(F) peak
Biomass Measurement	RUN	biomass Known(K) or Ignored(I)
Data Entry*	RUN	Transmittance(T) or Absorbance(A)
Comment	RUN	up to 20 characters long
Sample Identifier	SAMPLE	8 characters long
Dilution Factor	SAMPLE	---
Sample Volume/Area**	REPLICATE	liters (for plankton) m^2 (for periphyton)
Biomass Concentration	REPLICATE	mg/liter (for plankton) g/m^2 (for periphyton)

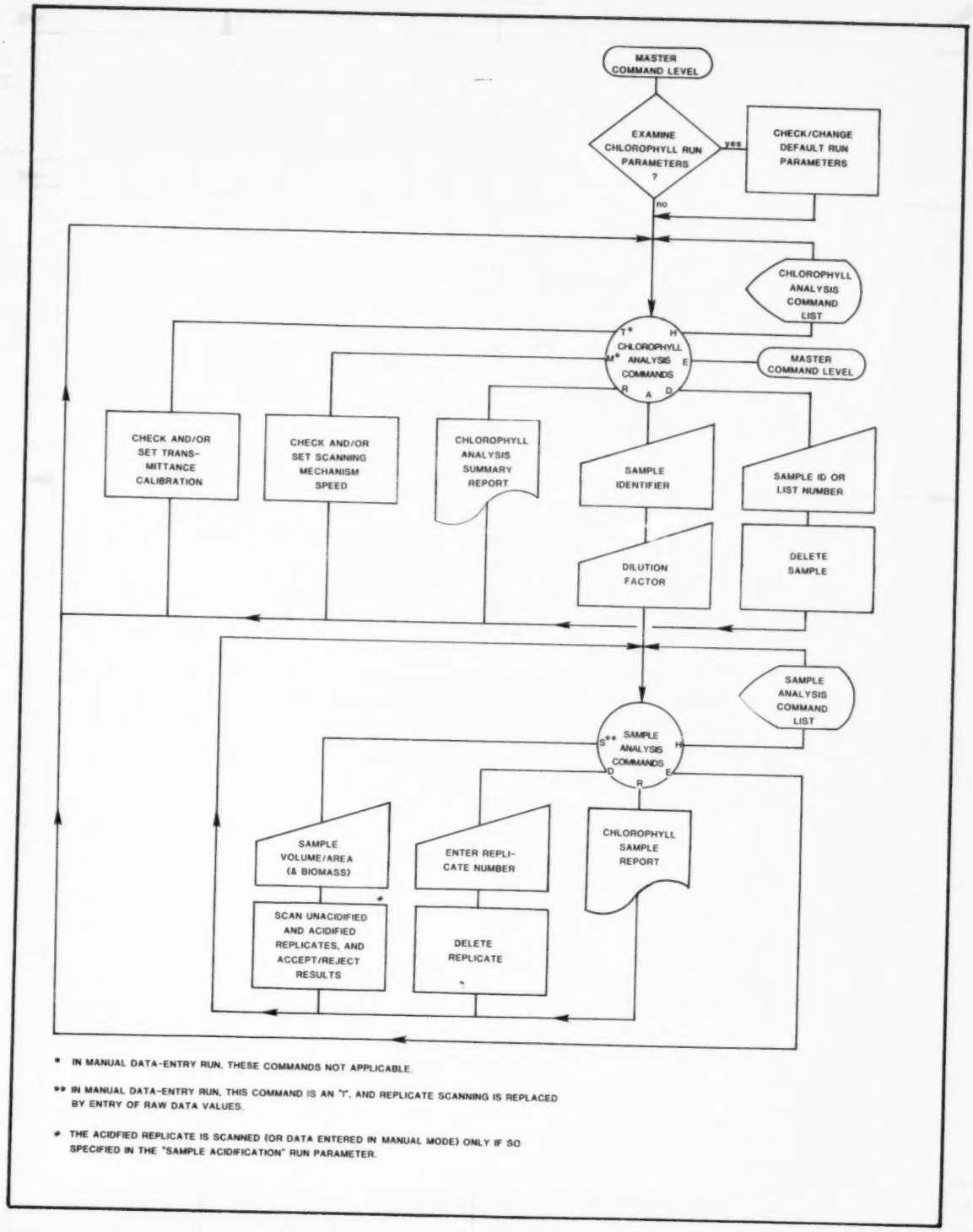
* Applies only to manual data-entry chlorophyll run.

** Sample volume/area will be same for all replicates, and is effectively a SAMPLE parameter in scope if all replicates are from same extract (as in Figure 7).

```
STATION/STUDY :                              LITTLE  MIAMI #1
        [KEEP(CR) OR CHANGE]  >>> LITTLE MIAMI #2↵

STATION/STUDY :                              LITTLE MIAMI #2
        [KEEP(CR) OR CHANGE]  >>> ↵
SAMPLE TYPE: PLANKTON [P] OR PERIPHYTON [E] : P
        [KEEP(CR) OR CHANGE]  >>> ↵
EXTRACT VOLUME (ML) :                        1500
        [KEEP(CR) OR CHANGE]  >>> 1000↵
EXTRACT VOLUME (ML) :                        1000
        [KEEP(CR) OR CHANGE]  >>> ↵
EXTRACTION METHOD :                          GRIND
        [KEEP(CR) OR CHANGE]  >>> ↵
CELL PATH LENGTH (CM) :                      1
        [KEEP(CR) OR CHANGE]  >>> ↵
COMPUTATION METHOD [U,S,J] :                 U
        [KEEP(CR) OR CHANGE]  >>> ↵
SAMPLES ACIDIFIED [A] OR NOT [N] : N
        [KEEP(CR) OR CHANGE]  >>> A↵
SAMPLES ACIDIFIED [A] OR NOT [N] : A
        [KEEP(CR) OR CHANGE]  >>> ↵
METHOD [M] OR FOUND [F] PEAK ABS : M
        [KEEP(CR) OR CHANGE]  >>> F↵
METHOD [M] OR FOUND [F] PEAK ABS : F
        [KEEP(CR) OR CHANGE]  >>> ↵
BIOMASS KNOWN [K] OR IGNORED [I] : I
        [KEEP(CR) OR CHANGE]  >>> ↵
DATA ENTRY: ABSORB.[A] OR %-TRANS.[T] : A
        [KEEP(CR) OR CHANGE]  >>> ↵
COMMENT :                                    STUDY  DATE: 7/23/80
        [KEEP(CR) OR CHANGE]  >>> STUDY DATE: 7/24/80↵
COMMENT :                                    STUDY DATE: 7/24/80
        [KEEP(CR) OR CHANGE]  >>> ↵
```

NOTE: CURVED ARROW MARKS WHERE USER PRESSES CARRIAGE RETURN; AND UNDERLINE SHOWS WHAT IS ENTERED BY USER.

Figure 11. An example of examining/changing chlorophyll run parameters.



* IN MANUAL DATA-ENTRY RUN, THESE COMMANDS NOT APPLICABLE.

** IN MANUAL DATA-ENTRY RUN, THIS COMMAND IS AN "I", AND REPLICATE SCANNING IS REPLACED BY ENTRY OF RAW DATA VALUES.

\# THE ACIDFIED REPLICATE IS SCANNED (OR DATA ENTERED IN MANUAL MODE) ONLY IF SO SPECIFIED IN THE "SAMPLE ACIDIFICATION" RUN PARAMETER.

Figure 12. Flow of operation of the UVVIS chlorophyll analysis.

TABLE 4. AUTOMATED CHLOROPHYLL ANALYSIS SUMMARY

```
+ OPTIONAL TASK:  ACCEPTANCE/ALTERATION OF DEFAULT CHLOROPHYLL RUN PARAMETERS;
+ CHLOROPHYLL ANALYSIS COMMANDS:

    T -- TRANSMITTANCE CALIBRATION CHECK/SET:
            + TASK:  ADJUSTMENT OF SPECTROPHOTOMETER AT 0% AND 100%
                     TRANSMITTANCE LEVELS;
            + OPTIONS:  1. CHECK OF CALIBRATION;
                        2. CALIBRATION AT 0% AND 100% TRANSMITTANCE;
                        3. CONTINUATION OF ANALYSIS SESSION (DEFAULT OPTION).

    M -- SCANNING MECHANISM SPEED CHECK/SET:
            OPTIONS:  1. CHECK OF PRESENT SPEED;
                      2. SETTING OF SPEED;
                      3. CONTINUATION OF ANALYSIS SESSION (DEFAULT OPTION).

    R -- SUMMARY CHLOROPHYLL REPORT:
            OPTIONS:  1. TERMINAL DISPLAY OF REPORT;
                      2. LINEPRINTER COPY OF REPORT;
                      3. CONTINUATION OF ANALYSIS SESSION (DEFAULT OPTION).

    A -- ANALYSIS OF ONE SAMPLE:
            + TASK:  ENTRY OF SAMPLE IDENTIFIER (OR ACCEPTANCE OF COMPUTER-DEFINED IDENTIFIER);
            + TASK:  ENTRY OF DILUTION FACTOR (OR ACCEPTANCE OF DEFAULT VALUE);
            + SAMPLE ANALYSIS COMMANDS:

                S -- SCAN OF ONE CHLOROPHYLL SAMPLE REPLICATE:
                        + TASK:  ENTRY OF SAMPLE VOLUME/AREA (OR ACCEPTANCE OF DEFAULT VALUE);
                        + TASK:  ENTRY OF BIOMASS (OR ACCEPTANCE OF DEFAULT VALUE);
                        + PROCESS:  FOR UNACIDIFIED (AND ACIDIFIED, IF DONE) SAMPLE:
                            + TASK:  PREPARATION/(ACIDIFICATION) OF SAMPLE;
                            + TASK:  PLACEMENT OF SAMPLE CUVETTE IN SPECTROPHOTOMETER CHAMBER;
                            + TASK:  SETTING OF WAVELENGTH DIAL AND INITIATION OF SCAN
                                     BY PRESSING RETURN KEY;
                            + INFORMATION:  TERMINAL DISPLAY OF SCAN RESULTS;
                            + DECISION:  ACCEPTANCE (DEFAULT ANSWER) OR REJECTION
                                         (RESCAN SAMPLE) OF RESULTS.

                D -- DELETION OF DATA OF ONE CHLOROPHYLL SAMPLE REPLICATE:
                        + TASK:  ENTRY OF REPLICATE NUMBER;
                        + INFORMATION:  TERMINAL DISPLAYED MESSAGE OF DELETION.

                R -- INTERMEDIATE SAMPLE REPORT:
                        OPTIONS:  1. TERMINAL DISPLAY OF REPORT;
                                  2. LINEPRINTER COPY OF REPORT;
                                  3. CONTINUATION OF FILTER ANALYSIS (DEFAULT OPTION).

                E -- END OF ANALYSIS OF THIS SAMPLE; RETURN TO CHLOROPHYLL ANALYSIS COMMAND LEVEL.

                H -- HELP:  TERMINAL DISPLAY OF SAMPLE ANALYSIS COMMAND EXPLANATIONS (DEFAULT COMMAND).

    D -- DELETION OF DATA OF ONE CHLOROPHYLL SAMPLE:
            OPTIONS:  1. TERMINAL DISPLAYED LISTING OF SAMPLES ANALYZED DURING SESSION;
                      2. DELETION BY SAMPLE IDENTIFIER:
                          + TASK:  ENTRY OF SAMPLE IDENTIFIER;
                          + INFORMATION:  TERMINAL DISPLAYED MESSAGE OF DELETION;
                          + CONTINUATION OF ANALYSIS SESSION.
                      3. DELETION BY LIST NUMBER (FROM OPTION 1 LIST):
                          + TASK:  ENTRY OF OPTION 1 LIST NUMBER;
                          + INFORMATION:  TERMINAL DISPLAYED MESSAGE OF DELETION;
                          + CONTINUATION OF ANALYSIS SESSION.
                      4. CONTINUATION OF ANALYSIS SESSION (DEFAULT OPTION).

    E -- END OF CHLOROPHYLL ANALYSIS SESSION; RETURN TO UVVIS MASTER COMMAND LEVEL.

    H -- HELP:  TERMINAL DISPLAY OF CHLOROPHYLL ANALYSIS COMMAND EXPLANATIONS (DEFAULT COMMAND).
```

TABLE 5. MANUAL DATA-ENTRY CHLOROPHYLL ANALYSIS SUMMARY

+ OPTIONAL TASK: ACCEPTANCE/ALTERATION OF DEFAULT CHLOROPHYLL RUN PARAMETERS;
+ CHLOROPHYLL ANALYSIS COMMANDS:

R — SUMMARY CHLOROPHYLL REPORT:
OPTIONS: 1. TERMINAL DISPLAY OF REPORT;
2. LINEPRINTER COPY OF REPORT;
3. CONTINUATION OF ANALYSIS SESSION (DEFAULT OPTION).

A — ANALYSIS OF ONE SAMPLE:
+ TASK: ENTRY OF SAMPLE IDENTIFIER (OR ACCEPTANCE OF COMPUTER-DEFINED IDENTIFIER);
+ TASK: ENTRY OF DILUTION FACTOR (OR ACCEPTANCE OF DEFAULT VALUE);
+ SAMPLE ANALYSIS COMMANDS:

I — INPUT OF DATA OF ONE CHLOROPHYLL SAMPLE REPLICATE:
+ TASK: ENTRY OF SAMPLE VOLUME/AREA (OR ACCEPTANCE OF DEFAULT VALUE);
+ TASK: ENTRY OF BIOMASS (OR ACCEPTANCE OF DEFAULT VALUE);
+ TASK: ENTRY OF RAW DATA (ABSORBANCE OR PERCENT TRANSMITTANCE VALUES) AT METHOD WAVELENGTHS FOR UNACIDIFIED (AND ACIDIFIED) SAMPLE;
+ INFORMATION: TERMINAL DISPLAY OF RESULTS;
+ DECISION: ACCEPTANCE (DEFAULT ANSWER) OR REJECTION (REENTER RAW DATA) OF RESULTS.

D — DELETION OF DATA OF ONE CHLOROPHYLL SAMPLE REPLICATE:
+ TASK: ENTRY OF REPLICATE NUMBER;
+ INFORMATION: TERMINAL DISPLAYED MESSAGE OF DELETION.

R — INTERMEDIATE SAMPLE REPORT:
OPTIONS: 1. TERMINAL DISPLAY OF REPORT;
2. LINEPRINTER COPY OF REPORT;
3. CONTINUATION OF FILTER ANALYSIS (DEFAULT OPTION).

E — END OF ANALYSIS OF THIS SAMPLE; RETURN TO CHLOROPHYLL ANALYSIS COMMAND LEVEL.

H — HELP: TERMINAL DISPLAY OF SAMPLE ANALYSIS COMMAND EXPLANATIONS (DEFAULT COMMAND).

D — DELETION OF DATA OF ONE CHLOROPHYLL SAMPLE:
OPTIONS: 1. TERMINAL DISPLAYED LISTING OF SAMPLES ANALYZED DURING SESSION;
2. DELETION BY SAMPLE IDENTIFIER:
+ TASK: ENTRY OF SAMPLE IDENTIFIER;
+ INFORMATION: TERMINAL DISPLAYED MESSAGE OF DELETION;
+ CONTINUATION OF ANALYSIS SESSION.
3. DELETION BY LIST NUMBER (FROM OPTION 1 LIST):
+ TASK: ENTRY OF OPTION 1 LIST NUMBER;
+ INFORMATION: TERMINAL DISPLAYED MESSAGE OF DELETION;
+ CONTINUATION OF ANALYSIS SESSION.
4. CONTINUATION OF ANALYSIS SESSION (DEFAULT OPTION).

E — END OF CHLOROPHYLL ANALYSIS SESSION; RETURN TO UVVIS MASTER COMMAND LEVEL.

H — HELP: TERMINAL DISPLAY OF CHLOROPHYLL ANALYSIS COMMAND EXPLANATIONS (DEFAULT COMMAND).

SECTION 7

ANALYZING COLOR

The color analysis procedures of the UVVIS system assist you in evaluating the color of waters by one of two methods. The automated procedure employs a method, developed by the American Dye Manufacturers Institute (ADMI),[3] which has been adapted for automated spectrophotometry in the UVVIS system. The manual data-entry procedure operates in conjunction with a filter photometer and the Three-Filter Tristimulus method.[4]

The UVVIS color analyses permit you to study three types of samples: 1) update standards; 2) check standards; and 3) unknowns. A set of update standards consists of six platinum-cobalt samples[4] of defined intensity: 25, 50, 100, 150, 200, and 250 ADMI color units. The same concentrations apply to any check standard. Unknowns may be any water sample, and need not be of the usual color of most river and stream waters. During the course of a run you may obtain separate reports for the three types of samples. You may also request a general summary report, as well as a report which summarizes accumulated standards statistics. Raw plot data are available in the automated procedure. Example reports and plots are shown in Figures 13 through 20.

Before ever studying unknowns, you must use the system to create a standards curve by establishing a relationship between the ADMI values of the six standards and the corresponding "DE" numbers. The DE number is a measure of the difference between a sample's color and a "colorless" solution. The standards curve is calibrated and maintained through the analysis of update standards, and is challenged by analyzing check standards. An unknown, regardless of color, is evaluated in terms of its DE number, and then related to the standards curve for its effective ADMI value. Please realize that because the automated and manual data-entry procedures use two different instruments, you must establish and maintain a distinct standards curve for each of the two procedures.

THE AUTOMATED RUN

After selecting the L master command and preparing for an automated run, you will be presented with the color analysis commands. The flow of operation of the UVVIS color analysis is depicted in Figure 21. The commands and options of the automated and manual data-entry analyses are summarized in Tables 6 and 7.

The Color Analysis Commands

The computer displays the following command line:

COLOR ANALYSIS COMMANDS: T, M, RG, RS, AS, AC, AU, E, H(CR) >>>

The default H command produces brief command explanations.

The T command enables you to check the present calibration and/or establish a fresh calibration throughout the scan range (400 to 700 nanometers). The most common reference medium used in color evaluation is distilled water. The procedures for checking and/or setting the transmittance calibration are described in Appendix B. You must set the speed of the scanning mechanism before the first transmittance calibration is ever done. Should it not be defined, then an appropriate message is displayed at your terminal, and the T command not processed.

The M command is for checking and/or setting the scanning mechanism speed. Any time you wish to change speeds, you must use this command. Consult Appendix C for details on this operation.

The RG and RS commands direct the system to produce reports. RG is for a general summary report, as shown in Figure 20, which lists basic results of check standards, and unknowns analyzed during the run, as well as data of the most recent update standards. RS yields a report which summarizes data and dates of all present sets of standards, as exemplified in Figure 19. See Appendix D for a few words on obtaining terminal and/or lineprinter reports.

The AS, AC, and AU commands are for sample analysis. AS permits you to add a set of update standards. AC is for check standards, while AU is for unknowns. The operation of these three commands is described below in Analyzing the Color Samples.

An E command notifies the system that you have finished the run. It then rises to master command level and presents to you the master commands.

THE MANUAL DATA-ENTRY RUN

After selecting the LM master command, you will be offered the color analysis commands. The flow of operation of the UVVIS color system analysis is depicted in Figure 21.

The Color Analysis Commands

The computer displays the following command line:

COLOR ANALYSIS COMMANDS: RG, RS, AS, AC, AU, E, H(CR) >>>

The default H command yields brief command explanations.

The remaining six commands (RG, RS, AS, AC, AU, and E) operate as in the automated run. Consult the Automated Run section above.

ANALYZING THE COLOR SAMPLES

In either the automated or manual data-entry runs, the AS, AC, and AU commands operate as described in the respective portions below.

Update Standards

After entering an AS command, you will have to wait for the system to prepare for the standards analysis. From that point on you will analyze each update standard, beginning at ADMI 25, and moving sequentially up through ADMI 250.

For each update standard, you will be told either to prepare it for scanning (in the automated mode) or to enter photometer data (in the manual data-entry mode). In an automated run, the computer will tell you to set the spectrophotometer for scanning at 705 nanometers, and to press the RETURN key. The computer will start the scan and collect the data. In a manual data-entry run, you will be asked to enter the three output data from the red, green, and blue filters, respectively in the form of percent transmittance.

Once the raw data have been collected by scanning or data entry, the system will compute the results and concisely display them to your terminal. You will be asked:

```
REJECT RESULTS OF ADMI XXX SCAN ?   N(CR) OR Y   >>>
```

where XXX is the ADMI value of the update standard. If you reject the results, then the computer will repeat its request for scanning/data-entry. Otherwise, you will proceed to the next standard.

Once all six standards have been studied, you may, if you wish, obtain a report which summarizes evaluations of the update standards. An example report is shown in Figure 13. (See Appendix D on Obtaining Reports.) Whether or not you want the report, you will then be asked:

```
REJECT UPDATE STANDARDS ?   N(CR) OR Y   >>>
```

Type an N (or just the RETURN key) to accept the set of update standard results. Only the Y response will cause a rejection of the results.

Should you accept their results, the system will add the results to the other standards, and will modify the standards curve. Realize that whenever you run a set of update standards after you have studied some check standards and/or unknowns, then those samples will be evaluated in terms of the new standards curve.

Whether or not you accept the update standards, the system will again offer you the color analysis commands.

Check Standards

An AC command initiates the study of check standards. Any number of them may be analyzed, but because the system does not recognize replicates, none of the data can be or need be deleted during the course of the run. Before processing a check standard, you may need to wait for the system to ready itself for the analysis. You must then assign the check standards identifier, and define its expected ADMI.

When assigning the check standard identifier, you will first be asked:

CHECK STANDARD ID: [USER-SET OR AUTOMATIC(CR)] >>>

By pressing the RETURN key, you let the system create an eight character ID of the form "Cmmddnnn," where mm is the month date, dd is the day date, and nnn counts the check standards. For example, the second check standard of a run made on July 23rd would be identified as "C0723002." If you make up your own identifier, it may be as many as eight characters long. An ID less than eight characters in length will be padded on the left with zeroes. Any blanks will be changed to zeroes. For example, were you to type in "CHECK 2," the sample would be identified as "0CHECK02." You must confirm or change the ID by answering the keep/change question:

CHECK STANDARD ID: XXXXXXXX [KEEP(CR) OR CHANGE] >>>

where XXXXXXXX is the identifier.

To define the expected ADMI of the color check standard, you must answer the following question:

SELECT CHECK STANDARD TYPE [1-6 OR H(CR)] >>>

An H (or simply RETURN key) will result in a list of ADMIs, numbered from one to six, where one corresponds to ADMI 25, and six corresponds to ADMI 250.

Once you have established the check standard's parameters, you must confirm the ADMI by answering the question:

CHECK STANDARD XXXXXXXX IS ADMI ZZZ. CHANGE ? [N(CR) OR Y] >>>

where XXXXXXXX is the identifier and ZZZ is the expected ADMI. The Y answer is available to allow you to correct any mistake.

You will then either have the sample scanned (in the automated mode) or enter the sample raw data (in the manual data-entry mode). In the automated run, you will be asked to set the spectrophotometer for scanning at 705 nanometers, and to press the RETURN key in order to start the scan and data collection. In the manual data-entry run, you will be requested to enter the three output data from the red, green, and blue filters, respectively in the form of percent transmittance.

Once the raw data have been collected, the system will compute the results and display them on your terminal. It will then ask:

```
REJECT RESULTS FOR CHECK STANDARD XXXXXXXX ?  [N(CR) OR Y]  >>>
```

where XXXXXXXX is the check standard ID. Should you reject the results, the process will start over, beginning with the definition of the ID and expected ADMI. Otherwise, you will be given three final options:

```
SELECT:
  ANOTHER CHECK STANDARD[1], CHECK STANDARDS REPORT[2], OR
  CONTINUE[3(CR)]  >>>
```

Option 1 is for analyzing the next check standard. Option 2 yields a report, as shown in Figure 15, which presents data for all check standards analyzed in the run. (See Appendix D on Obtaining Reports.) Under option 3, the system again offers you the color analysis commands.

Unknowns

An AU command initiates the study of an unknown, any number of which may be analyzed. Unknowns cannot and need not be deleted, since the system does not consider replicates. Before processing the unknown, you may need to wait for the system to ready itself for the analysis. You must then assign the unknown's identifier and may attach an applicable comment.

When assigning the unknown identifier, you will first be asked:

```
UNKNOWN ID:  [USER-SET OR AUTOMATIC(CR)]  >>>
```

By pressing the RETURN key, the system will create an eight character ID of the form "Ummddnnn," where mm is the month date, dd is the day date, and nnn counts the unknowns. For example, the third unknown of a run made on July 23rd would be identified as "U0723003." If you create the ID yourself, it may not exceed eight characters in length. An ID less than eight characters long will be padded on the left with zeroes, and any blanks will be changed to zeroes. For example, were you to type in "UN 3," the sample would be identified as "0000UN03." You must confirm or change the ID by answering the question:

```
UNKNOWN ID:  XXXXXXXX  [KEEP(CR) OR CHANGE]  >>>
```

where XXXXXXXX is the identifier.

To supply an accompanying comment for the unknown, you may answer the question:

```
COMMENT FOR UNKNOWN:        XXXXXXXXXXXXXXXXXXXXXXXXXXXXX
   [KEEP(CR) OR CHANGE]  >>>
```

where the X's are the comment of the directly preceeding unknown. The comment may not exceed thirty characters in length. Once the comment is defined, the procedure will continue.

You will then either have the sample scanned (in the automated mode) or enter the sample raw data (in the manual data-entry mode). In the automated run, you will be asked to set the spectrophotometer for scanning at 705 nanometers, and to press the RETURN key in order to start the scan and data collection. In the manual data-entry run, you will be requested to enter the three output data from the red, green, and blue filters, respectively in the form of percent transmittance.

One the raw data have been collected, the system will compute the results and display them on your terminal. It will then ask:

```
REJECT RESULTS FOR UNKNOWN XXXXXXXX ?  [N(CR) OR Y]  >>>
```

where XXXXXXXX is the unknown ID. Should you reject the results, the process will start over, beginning with the definition of the ID and comment. Otherwise, you will be given three final options:

```
SELECT:
  ANOTHER UNKNOWN[1], UNKNOWNS REPORT[2], OR CONTINUE[3(CR)]  >>>
```

Option 1 is for analyzing the next unknown. Option 2 yields a report, as exemplified in Figure 17, which presents data for all unknowns analyzed in the run. (See Appendix D on Obtaining Reports.) Under option 3, the system again offers you the color analysis commands.

UVVIS COLOR ANALYSIS
UPDATE STANDARDS REPORT

DATE/TIME: 8/18/80 2:26 P.M.

ANALYST: GREGORY S. ROTH INSTRUMENT: P.E. COLEMAN 124

STANDARDS CALIBRATION EQUATION —
ADMI = 2.080E+00 + (2.645E+02 * DE) + (5.476E+01 * DE^2)
[STANDARD ERROR OF ESTIMATE(SEE): 0.719 %REL SEE: 0.56]

ADMI OF APHA STANDARD	DE OF MOST RECENT UPDATE [A]	MEAN DE OF ALL STNDS. [B]	DE DIFFERENCE [A-B=D]	STANDARD DEV. OF MEAN DE	UPDATE CONTROL RATING
25	0.0835	0.0871	-0.0036	0.0008	***
50	0.1734	0.1731	0.0003	0.0013	*
100	0.3450	0.3439	0.0011	0.0012	*
150	0.5085	0.5068	0.0017	0.0031	*
200	0.6631	0.6606	0.0025	0.0042	*
250	0.8042	0.8022	0.0019	0.0048	*

NOTE: CONTROL RATINGS BASED ON ABSOLUTE VALUE(D') OF DE DIFF(D) RELATIVE TO STND DEV(S) OF STANDARDS MEAN DE, SUCH THAT: * — IN CONTROL [D'<2S]; ** — WARNING [2S<=D'<=3S]; *** — OUT OF CONTROL [D'>3S].

Figure 13. An example report for a set of color update standards.

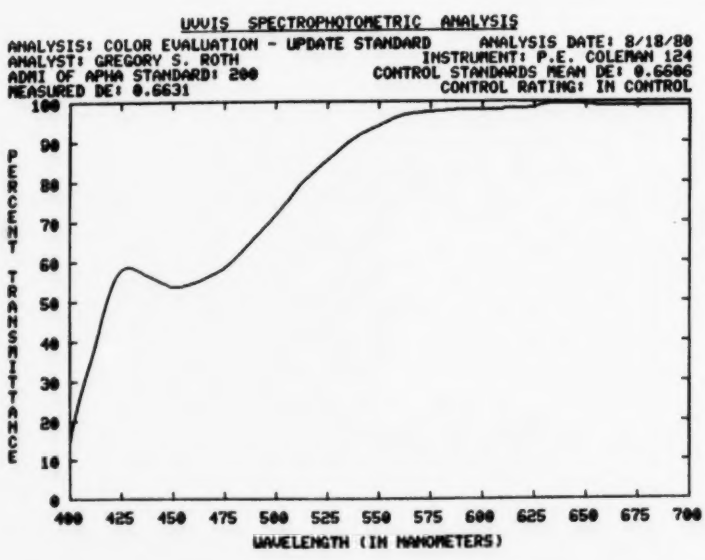
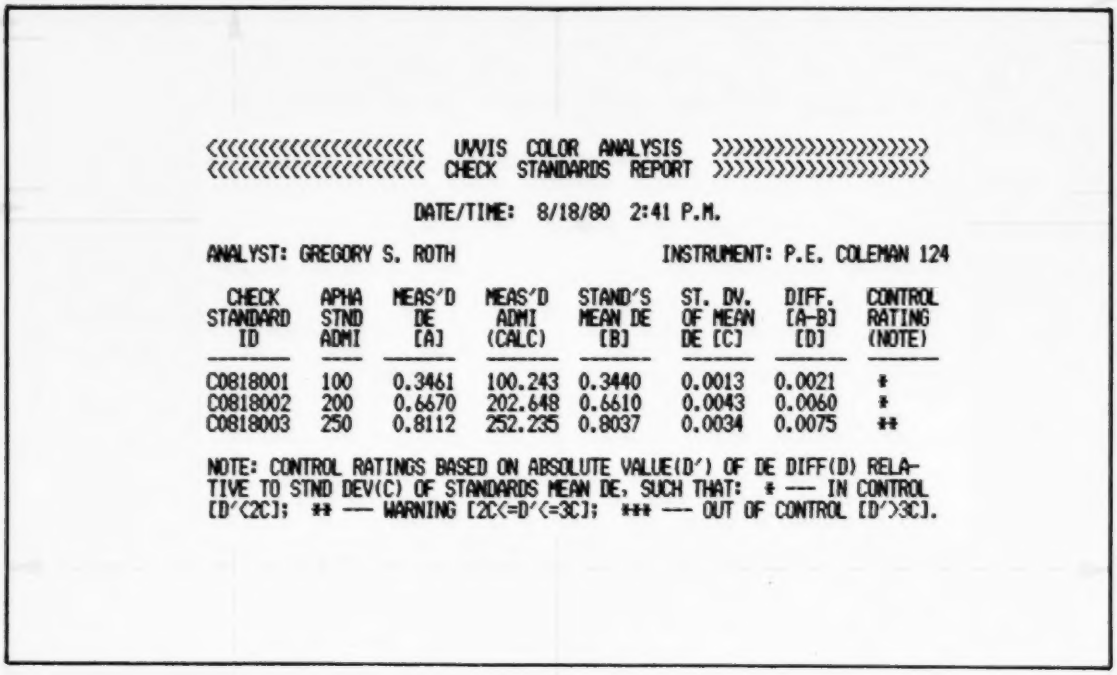


Figure 14. An example raw data plot of one color update standard.



UVVIS COLOR ANALYSIS
CHECK STANDARDS REPORT

DATE/TIME: 8/18/80 2:41 P.M.

ANALYST: GREGORY S. ROTH INSTRUMENT: P.E. COLEMAN 124

CHECK STANDARD ID	APHA STND ADMI	MEAS'D DE [A]	MEAS'D ADMI (CALC)	STAND'S MEAN DE [B]	ST. DV. OF MEAN DE [C]	DIFF. [A-B] [D]	CONTROL RATING (NOTE)
C0818001	100	0.3461	100.243	0.3440	0.0013	0.0021	*
C0818002	200	0.6670	202.648	0.6610	0.0043	0.0060	*
C0818003	250	0.8112	252.235	0.8037	0.0034	0.0075	**

NOTE: CONTROL RATINGS BASED ON ABSOLUTE VALUE(D') OF DE DIFF(D) RELATIVE TO STND DEV(C) OF STANDARDS MEAN DE, SUCH THAT: * --- IN CONTROL [D'<2C]; ** --- WARNING [2C<=D'<=3C]; *** --- OUT OF CONTROL [D'>3C].

Figure 15. An example report for color check standards.

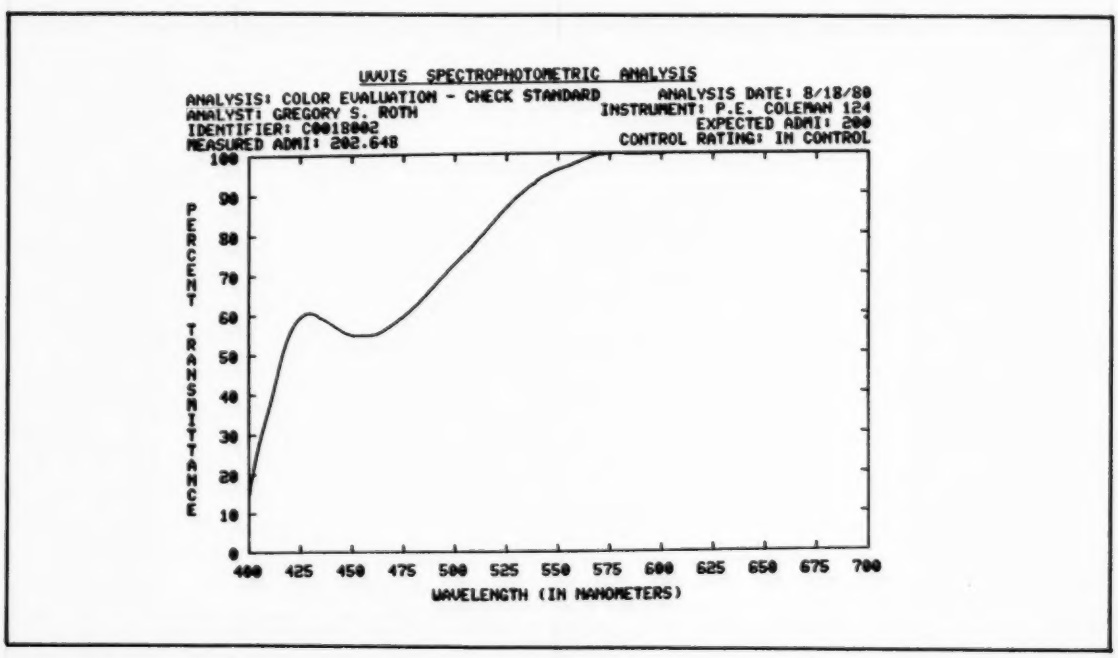


Figure 16. An example raw data plot of one color check standard.

UVVIS COLOR ANALYSIS
SAMPLE UNKNOWNS REPORT

DATE/TIME: 8/18/80 3:02 P.M.

ANALYST: GREGORY S. ROTH INSTRUMENT: P.E. COLEMAN 124

SAMPLE ID	MEASURED DE NUMBER	CALCULATED ADMI VALUE	SAMPLE COMMENT
U0818001	0.3285	94.944	ACID ORANGE 7
U0818002	0.3245	93.741	ACID BLUE 25
U0818003	0.3144	90.720	BASIC GREEN 4
U0818004	0.2461	70.572	BASIC VIOLET 1

Figure 17. An example report for color unknowns.

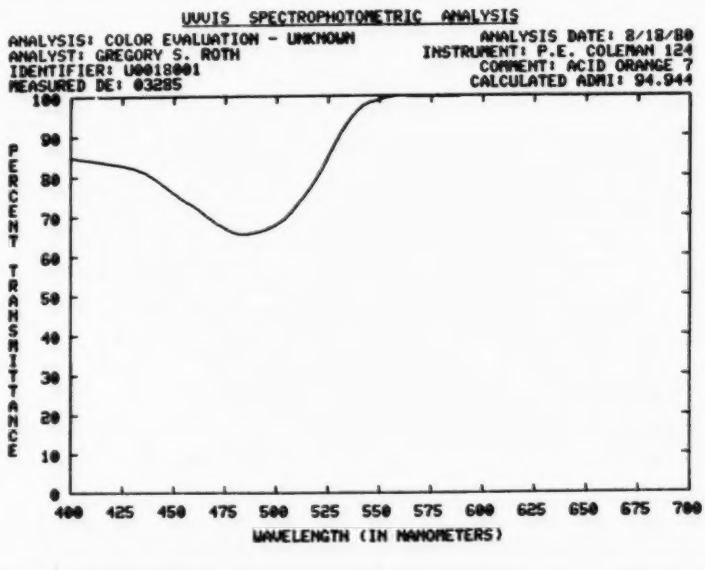
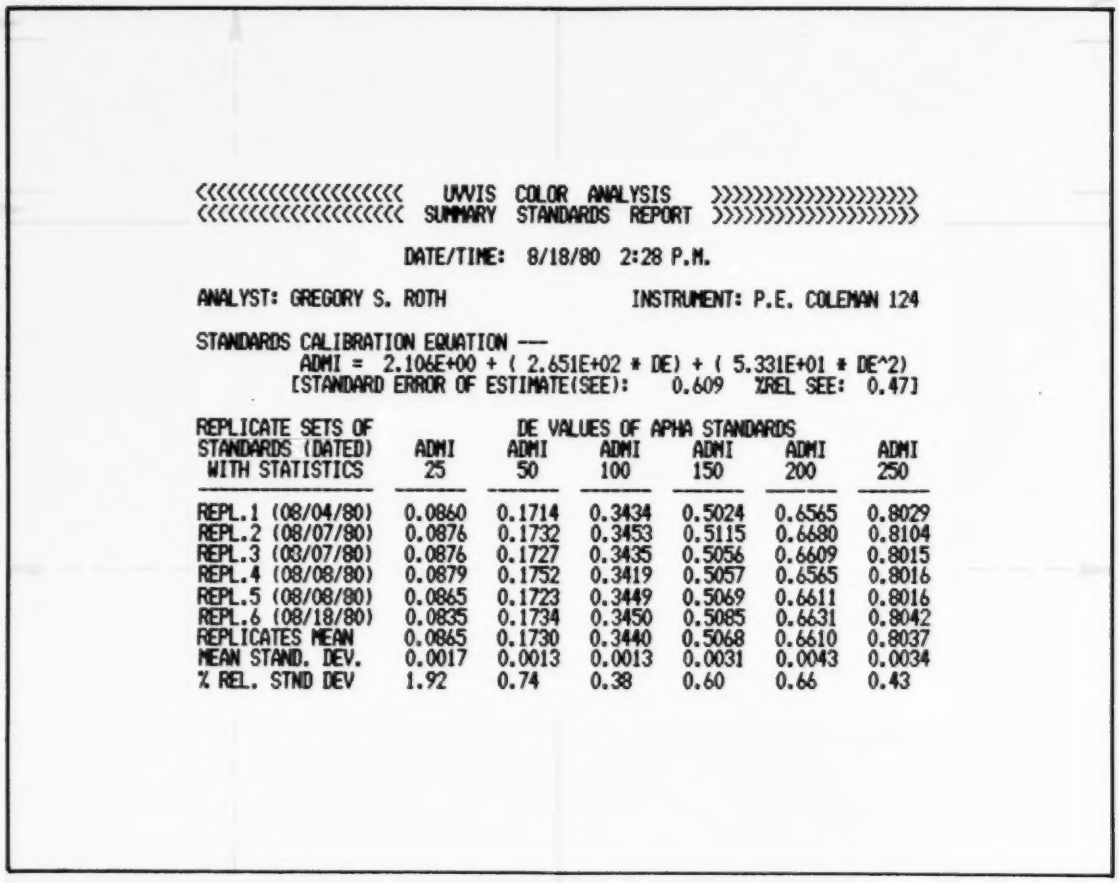


Figure 18. An example raw data plot of one color unknown.



UVVIS COLOR ANALYSIS
SUMMARY STANDARDS REPORT

DATE/TIME: 8/18/80 2:28 P.M.

ANALYST: GREGORY S. ROTH INSTRUMENT: P.E. COLEMAN 124

STANDARDS CALIBRATION EQUATION —
ADMI = 2.106E+00 + (2.651E+02 * DE) + (5.331E+01 * DE^2)
[STANDARD ERROR OF ESTIMATE(SEE): 0.609 %REL SEE: 0.47]

REPLICATE SETS OF STANDARDS (DATED) WITH STATISTICS	DE VALUES OF APHA STANDARDS ADMI 25	ADMI 50	ADMI 100	ADMI 150	ADMI 200	ADMI 250
REPL.1 (08/04/80)	0.0860	0.1714	0.3434	0.5024	0.6565	0.8029
REPL.2 (08/07/80)	0.0876	0.1732	0.3453	0.5115	0.6680	0.8104
REPL.3 (03/07/80)	0.0876	0.1727	0.3435	0.5056	0.6609	0.8015
REPL.4 (08/08/80)	0.0879	0.1752	0.3419	0.5057	0.6565	0.8016
REPL.5 (08/08/80)	0.0865	0.1723	0.3449	0.5069	0.6611	0.8016
REPL.6 (08/18/80)	0.0835	0.1734	0.3450	0.5085	0.6631	0.8042
REPLICATES MEAN	0.0865	0.1730	0.3440	0.5068	0.6610	0.8037
MEAN STAND. DEV.	0.0017	0.0013	0.0013	0.0031	0.0043	0.0034
% REL. STND DEV	1.92	0.74	0.38	0.60	0.66	0.43

Figure 19. An example summary report for color standards.

UVVIS

COLOR ANALYSIS SUMMARY

DATE/TIME: 8/18/80 3:05 P.M.

ANALYST: GREGORY S. ROTH INSTRUMENT: P.E. COLEMAN 124

STANDARDS INFORMATION:

CALIBRATION EQUATION —

ADMI = 2.106E+00 + (2.651E+02 * DE) + (5.331E+01 * DE^2)

[STANDARD ERROR OF ESTIMATE(SEE): 0.000 %REL SEE: 0.00]

MOST RECENT STANDARDS UPDATE: 8/18/80

ADMI OF APHA STANDARD	MEAN DE OF ALL STANDARDS	STANDARD DEVIATION OF MEAN DE	% RELATIVE STND. DEV. OF MEAN DE
25	0.0865	0.0017	1.925
50	0.1730	0.0013	0.737
100	0.3440	0.0013	0.380
150	0.5068	0.0031	0.605
200	0.6610	0.0043	0.656
250	0.8037	0.0034	0.429

CHECK STANDARDS INFORMATION:

CHECK STANDARD ID	APHA STND ADMI	MEAS'D DE [A]	MEAS'D ADMI (CALC)	STAND'S MEAN DE [B]	ST. DV. OF MEAN DE [C]	DIFF. [A-B] [D]	CONTROL RATING (NOTE)
C0818001	100	0.3461	100.243	0.3440	0.0013	0.0021	*
C0818002	200	0.6670	202.648	0.6610	0.0043	0.0060	*
C0818003	250	0.8112	252.235	0.8037	0.0034	0.0075	**

NOTE: CONTROL RATINGS BASED ON ABSOLUTE VALUE(D') OF DE DIFF(D) RELATIVE TO STND DEV(C) OF STANDARDS MEAN DE, SUCH THAT: * — IN CONTROL [D'<2C]; ** — WARNING [2C<=D'<=3C]; *** — OUT OF CONTROL [D'>3C].

UNKNOWNS INFORMATION:

SAMPLE ID	MEASURED DE NUMBER	CALCULATED ADMI VALUE	SAMPLE COMMENT
U0818001	0.3285	94.944	ACID ORANGE 7
U0818002	0.3245	93.741	ACID BLUE 25
U0818003	0.3144	90.720	BASIC GREEN 4
U0818004	0.2461	70.572	BASIC VIOLET 1

Figure 20. An example summary report for a color run.

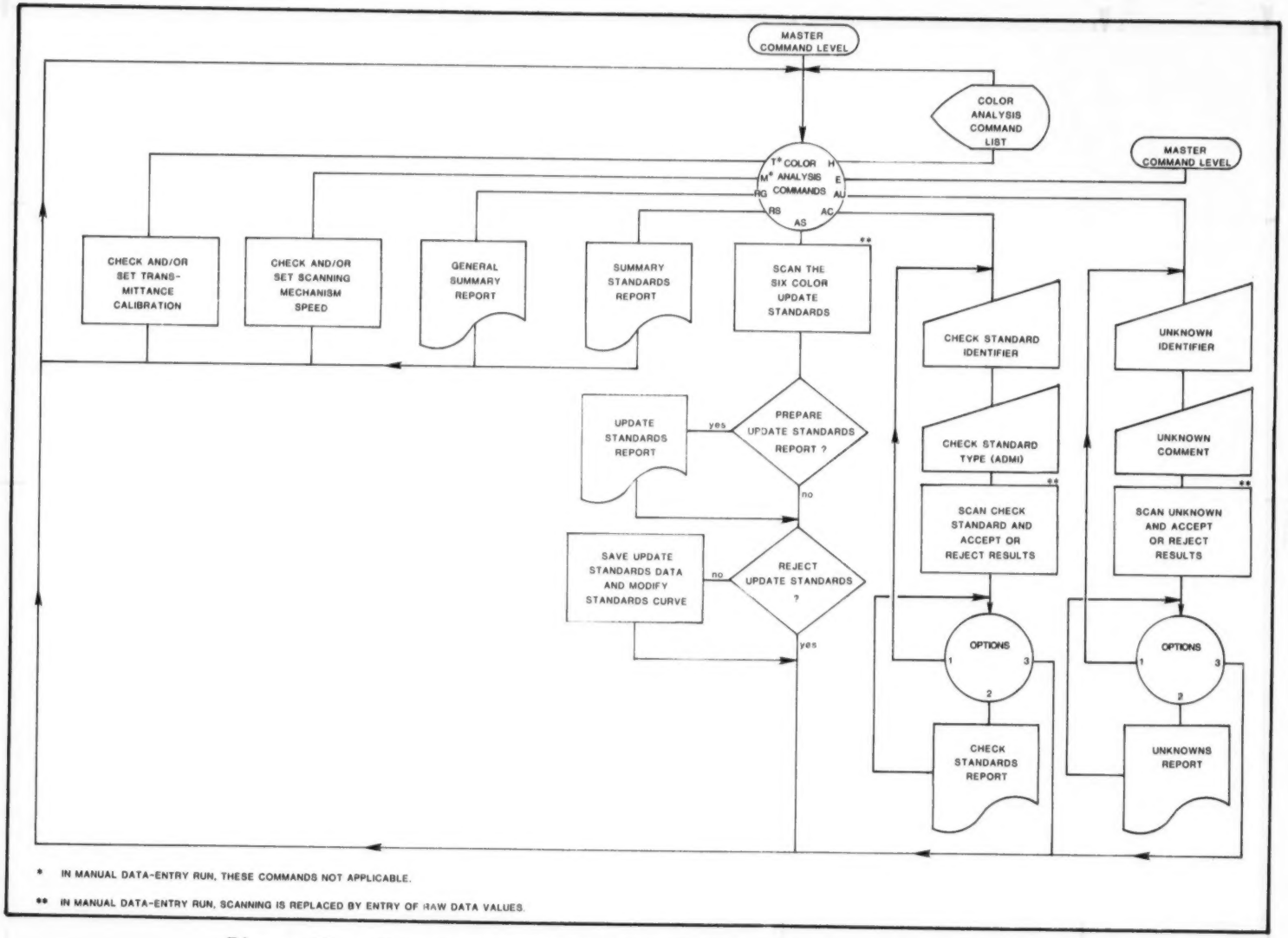


Figure 21. Flow of operation of the UVVIS color analysis.

TABLE 6. AUTOMATED COLOR ANALYSIS SUMMARY

COLOR ANALYSIS COMMANDS:

T ——— TRANSMITTANCE CALIBRATION CHECK/SET:
+ TASK: ADJUSTMENT OF SPECTROPHOTOMETER AT 0% AND 100% TRANSMITTANCE LEVELS;
+ OPTIONS: 1. CHECK OF CALIBRATION;
2. CALIBRATION AT 0% AND 100% TRANSMITTANCE;
3. CONTINUATION OF ANALYSIS SESSION (DEFAULT OPTION).

M ——— SCANNING MECHANISM SPEED CHECK/SET:
OPTIONS: 1. CHECK OF PRESENT SPEED;
2. SETTING OF SPEED;
3. CONTINUATION OF ANALYSIS SESSION (DEFAULT OPTION).

RG ——— GENERAL SUMMARY REPORT:
OPTIONS: 1. TERMINAL DISPLAY OF REPORT;
2. LINEPRINTER COPY OF REPORT;
3. CONTINUATION OF ANALYSIS SESSION (DEFAULT OPTION).

RS ——— SUMMARY STANDARDS REPORT:
OPTIONS: 1. TERMINAL DISPLAY OF REPORT;
2. LINEPRINTER COPY OF REPORT;
3. CONTINUATION OF ANALYSIS SESSION (DEFAULT OPTION).

AS ——— ANALYSIS OF ONE SET OF UPDATE STANDARDS:
+ PROCESS: FOR EACH STANDARD, BEGINNING WITH ADMI 25:
+ TASK: PLACEMENT OF STANDARD CUVETTE IN SPECTROPHOTOMETER CHAMBER;
+ TASK: SETTING OF WAVELENGTH DIAL AND INITIATION OF SCAN BY PRESSING RETURN KEY;
+ INFORMATION: TERMINAL DISPLAY OF RESULTS FOR STANDARD;
+ DECISION: ACCEPTANCE (DEFAULT ANSWER) OR REJECTION (RESCAN SAME STANDARD) OF RESULTS.
+ DECISION: BYPASS (DEFAULT ANSWER) OR PREPARATION OF UPDATE STANDARDS REPORT; IF PREPARED, OPTIONS:
1. TERMINAL DISPLAY OF REPORT;
2. LINEPRINTER COPY OF REPORT;
3. CONTINUATION TO NEXT DECISION (DEFAULT OPTION).
+ DECISION: ACCEPTANCE (DEFAULT ANSWER) OR REJECTION OF NEW SET OF UPDATE STANDARDS; IF ACCEPTED, INFORMATION:
TERMINAL DISPLAYED MESSAGE OF STANDARDS UPDATE.
+ CONTINUATION OF ANALYSIS SESSION; RETURN TO COLOR ANALYSIS COMMAND LEVEL.

AC/AU — ANALYSIS OF CHECK STANDARDS (CS) / ANALYSIS OF UNKNOWNS:
+ TASK: ENTRY OF CS/UNKNOWN IDENTIFIER (OR ACCEPTANCE OF COMPUTER-DEFINED IDENTIFIER);
+ TASK: FOR CS ONLY, SELECTION OF CHECK STANDARD TYPE (I.E. ADMI VALUE);
+ TASK: FOR UNKNOWN ONLY, ENTRY OF COMMENT PERTAINING TO UNKNOWN;
+ TASK: PLACEMENT OF CS/UNKNOWN CUVETTE IN SPECTROPHOTOMETER CHAMBER;
+ TASK: SETTING OF WAVELENGTH DIAL AND INITIATION OF SCAN BY PRESSING RETURN KEY;
+ INFORMATION: TERMINAL DISPLAY OF RESULTS FOR CS/UNKNOWN;
+ DECISION: ACCEPTANCE (DEFAULT ANSWER) OR REJECTION OF RESULTS;
+ OPTIONS: 1. ANALYSIS OF ANOTHER CS/UNKNOWN;
2. PREPARATION OF CS/UNKNOWNS REPORT:
OPTIONS: (1) TERMINAL DISPLAY OF REPORT;
(2) LINEPRINTER COPY OF REPORT;
(3) CONTINUATION OF ANALYSIS SESSION (DEFAULT OPTION); RETURN TO CS/UNKNOWNS OPTIONS.
3. CONTINUATION OF ANALYSIS SESSION (DEFAULT OPTION); RETURN TO COLOR ANALYSIS COMMAND LEVEL.

E ——— END OF COLOR ANALYSIS SESSION; RETURN TO UVVIS MASTER COMMAND LEVEL.

H ——— HELP: TERMINAL DISPLAY OF COLOR ANALYSIS COMMAND EXPLANATIONS (DEFAULT COMMAND).

TABLE 7. MANUAL DATA-ENTRY COLOR ANALYSIS SUMMARY

COLOR ANALYSIS COMMANDS:

RG ----- GENERAL SUMMARY REPORT:
OPTIONS: 1. TERMINAL DISPLAY OF REPORT;
2. LINEPRINTER COPY OF REPORT;
3. CONTINUATION OF ANALYSIS SESSION (DEFAULT OPTION).

RS ----- SUMMARY STANDARDS REPORT:
OPTIONS: 1. TERMINAL DISPLAY OF REPORT;
2. LINEPRINTER COPY OF REPORT;
3. CONTINUATION OF ANALYSIS SESSION (DEFAULT OPTION).

AS ----- ANALYSIS OF ONE SET OF UPDATE STANDARDS:
+ PROCESS: FOR EACH STANDARD, BEGINNING WITH ADMI 25:
+ TASK: PLACEMENT OF STANDARD CUVETTE IN FILTER PHOTOMETER;
+ TASK: ENTRY OF PERCENT "TRANSMITTANCE" VALUES FOR RED, BLUE AND GREEN PHOTOMETER FILTERS, RESPECTIVELY;
+ INFORMATION: TERMINAL DISPLAY OF RESULTS FOR STANDARD;
+ DECISION: ACCEPTANCE (DEFAULT ANSWER) OR REJECTION (REENTER DATA OF SAME STANDARD) OF RESULTS.
+ DECISION: BYPASS (DEFAULT ANSWER) OR PREPARATION OF UPDATE STANDARDS REPORT; IF PREPARED, OPTIONS:
1. TERMINAL DISPLAY OF REPORT;
2. LINEPRINTER COPY OF REPORT;
3. CONTINUATION TO NEXT DECISION (DEFAULT OPTION).
+ DECISION: ACCEPTANCE (DEFAULT ANSWER) OR REJECTION OF NEW SET OF UPDATE STANDARDS; IF ACCEPTED, INFORMATION:
TERMINAL DISPLAYED MESSAGE OF STANDARDS UPDATE.
+ CONTINUATION OF ANALYSIS SESSION; RETURN TO COLOR ANALYSIS COMMAND LEVEL.

AC/AU -- ANALYSIS OF CHECK STANDARDS (CS) / ANALYSIS OF UNKNOWNS:
+ TASK: ENTRY OF CS/UNKNOWN IDENTIFIER (OR ACCEPTANCE OF COMPUTER-DEFINED IDENTIFIER);
+ TASK: FOR CS ONLY, SELECTION OF CHECK STANDARD TYPE (I.E. ADMI VALUE);
+ TASK: FOR UNKNOWN ONLY, ENTRY OF COMMENT PERTAINING TO UNKNOWN;
+ TASK: PLACEMENT OF CS/UNKNOWN CUVETTE IN FILTER PHOTOMETER;
+ TASK: ENTRY OF PERCENT "TRANSMITTANCE" VALUES FOR RED, BLUE AND GREEN PHOTOMETER FILTERS, RESPECTIVELY;
+ INFORMATION: TERMINAL DISPLAY OF RESULTS FOR CS/UNKNOWN;
+ DECISION: ACCEPTANCE (DEFAULT ANSWER) OR REJECTION OF RESULTS;
+ OPTIONS: 1. ANALYSIS OF ANOTHER CS/UNKNOWN;
2. PREPARATION OF CS/UNKNOWNS REPORT:
OPTIONS: (1) TERMINAL DISPLAY OF REPORT;
(2) LINEPRINTER COPY OF REPORT;
(3) CONTINUATION OF ANALYSIS SESSION (DEFAULT OPTION); RETURN TO CS/UNKNOWNS OPTIONS.
3. CONTINUATION OF ANALYSIS SESSION (DEFAULT OPTION); RETURN TO COLOR ANALYSIS COMMAND LEVEL.

E ------ END OF COLOR ANALYSIS SESSION; RETURN TO UVVIS MASTER COMMAND LEVEL.

H ------ HELP: TERMINAL DISPLAY OF COLOR ANALYSIS COMMAND EXPLANATIONS (DEFAULT COMMAND).

SECTION 8

PLOTTING THE AUTOMATED CHLOROPHYLL/COLOR ANALYSIS RAW SCAN DATA

The UVVIS plotting function is accessed either as a master command of the UVVIS system, or independently under the UVVIS plot ID. The flow of operation of the UVVIS plotting function is depicted in Figure 22. Before plotting, you must examine the current (default) plot factors ("parameters").

The initial parameter is the type of terminal you have. You are given the following keep/change question:

```
TERMINAL TYPE -- PLOTTING[P] OR NON-PLOTTING[N] : X
                          [KEEP(CR) OR CHANGE]  >>>
```

where X is the present default terminal type P or N.

In the case of a plotting terminal, you must set two other parameters. One is the terminal make, having the question:

```
TERMINAL MAKE -- HEWLETT-PACKARD[H] OR TEKTRONIX [T] : X
                 [KEEP(CR) OR CHANGE]  >>>
```

where X is the present default terminal make H or T. The other parameter to be examined is the grid overlay feature, with the questions:

```
GRID OVERLAY -- NEVER [N], ALWAYS [A], OR PER PLOT [P] : X
                [KEEP(CR) OR CHANGE]  >>>
```

where X is the present default option N, A, or P. The grid overlay is exhibited in Figure 23.

In the case of a non-plotting terminal, you must first decide to have all plot curves formatted in the same nanometer increment, or in a different increment each plot curve. You will be asked:

```
LISTING NM. INC. -- SAME ALL PLOTS [S] OR PER PLOT [P] : X
                    [KEEP(CR) OR CHANGE]  >>>
```

where X is the default option S or P. Should you choose an S, you will be asked:

```
SET LISTING NANOMETER INCREMENT : XX
    [KEEP(CR) OR CHANGE] >>>
```

where XX is the current increment (1, 2, 5, or 10 nanometers). Accept the default or enter one of the other valid increments. A plot curve in ten nanometer increments is shown in Figure 24.

After defining the plot parameters, you will be offered the following commands:

```
PLOT COMMANDS:  C, L, E, H(CR)  >>>
```

H is the default command which lists command explanations. The C and L commands are for chlorophyll and color plots, respectively. The E command causes one of two events. If you are using the UVVIS system then it will return you to master command level. If you are using just the plot facility, then it will log you off from the Nova.

Under either the C or L commands, you will be given several options:

```
XX AVAILABLE PLOT(S) --
  DISPLAY INFO [I], PLOT  P  OR END PLOTTING [E(CR)]  >>>
```

where XX is the number of available chlorophyll/color plots. If you are using a "non-plotting" terminal, the second option (P) will be denoted as "LIST PLOT CURVE." Option I produces a numbered list of basic information for each plot. Option P creates the plots (or listed plot curves). The default option E again presents to you the plot commands.

Under either the I or P options, if there is more than one chlorophyll/color plot, then you will be asked an additional question:

```
PLOT NUMBERS  [1-XX]  :
```

where XX is the number of available chlorophyll/color plots. You may answer in one of two ways. Should you desire only one plot, merely enter its number. Should you want more than one, then enter the lowest number, followed directly by a dash, followed directly by the highest number. For example, suppose that there were ten available plots, and that you wanted information (I) concerning or plots (P) of the fifth through ninth plots. In this case, you would type "5-9", naturally followed by the RETURN key.

The listing of plot information or plotting of data will then proceed. In the case of multiple plots (or listed plot curves), you may ESCAPE around any of them. Considering the above example, suppose you wanted the fifth, sixth, seventh, and ninth plots. After the seventh plot, you would skip around the eighth plot by pressing the ESCAPE key. Because the ESCAPE key works in this particular manner when dealing with multiple plots, you may let any amount of data be prepared before escaping.

The UVVIS plotting function is designed so that if you should leave your terminal unattended, it will work its way to the E plot command. Each question is limited by time, and will answer itself if you do not answer it

within the time limit which is usually 45 or 60 seconds. This peculiarity has been implemented in consideration of an independent plotting terminal used by several people.

FOR PLOTTING TERMINALS

If in the plot parameters you have specified that you wish to decide about the grid overlay feature before each plot is made, then every plot will be preceeded by the question:

```
FOR PLOT XX:  USE GRID OVERLAY ? [N(CR) OR Y]  >>>
```

where XX is the number of the plot. By your entering an N (or just pressing the RETURN key) the computer will ignore the feature. Only a Y answer will ensure the grid overlay.

The plot heading information and data are then drawn on the screen. After a plot, you will have one minute in which to respond in some way. If the computer receives no response within that time frame, then it will proceed to the next plot. However, in the case of the final plot, it will again offer you the plot options (I, P and E).

FOR NON-PLOTTING TERMINALS

If in the plot parameters you have specified that you wish to define the listing nanometer increment before each curve is prepared, then every plot is preceeded by the question:

```
SET LISTING NANOMETER INCREMENT : XX
      [KEEP(CR) OR CHANGE]  >>>
```

where XX is the last increment you defined. Press the RETURN key to keep the default increment, or enter one of the other proper increments (1, 2, 5, or 10).

The plot is then prepared. A message concerning its preparation will be displayed on your terminal. You will then be given the terminal/lineprinter output options as described in Appendix D, Obtaining Reports. There is a one-minute time limit for choosing an output option. If the computer receives no response within that time frame, then it will continue to prepare the next plot. However, in the case of the last plot, it will again present to you the plot options (I, P and E).

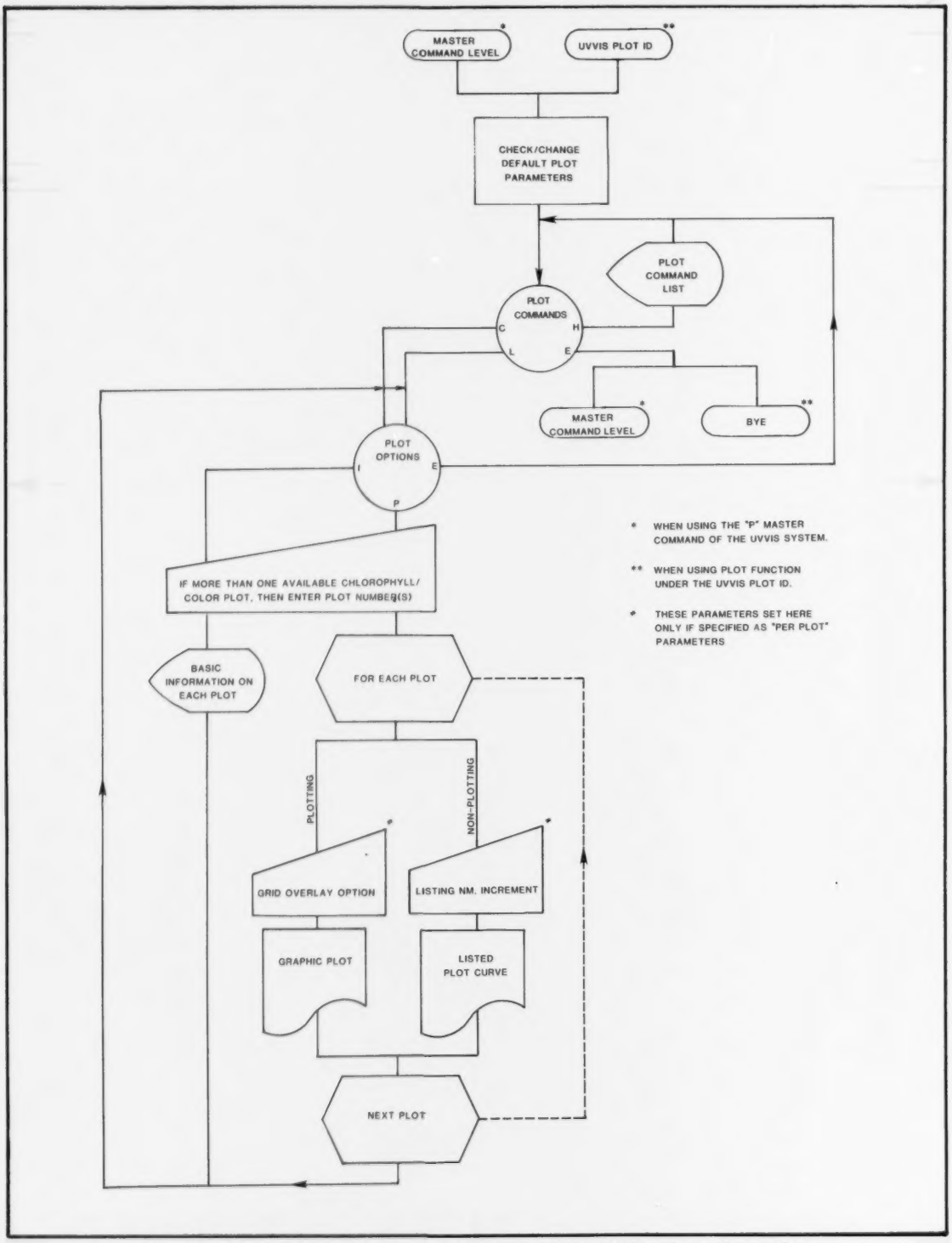


Figure 22. Flow of operation of the UVVIS plot function.

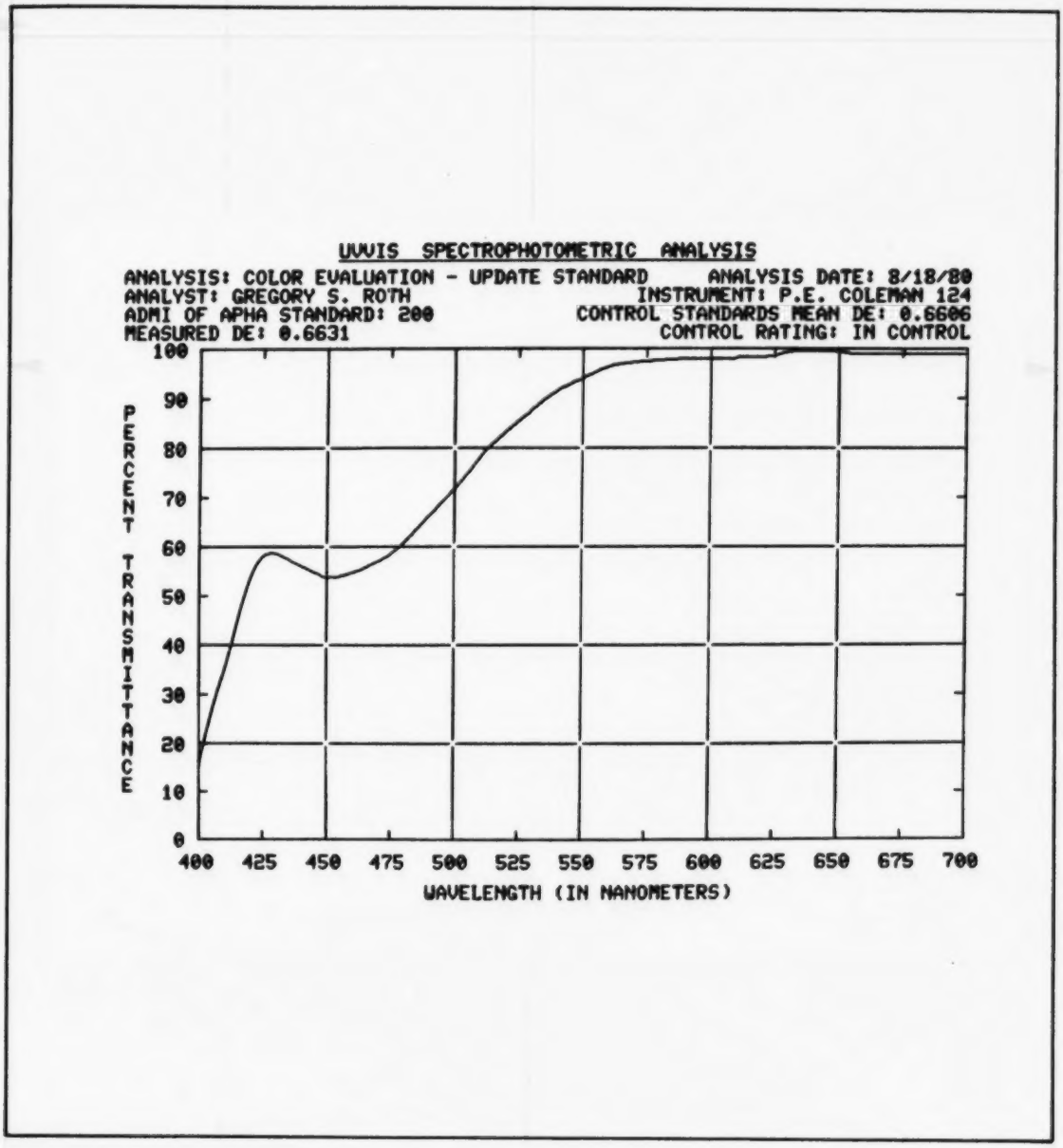
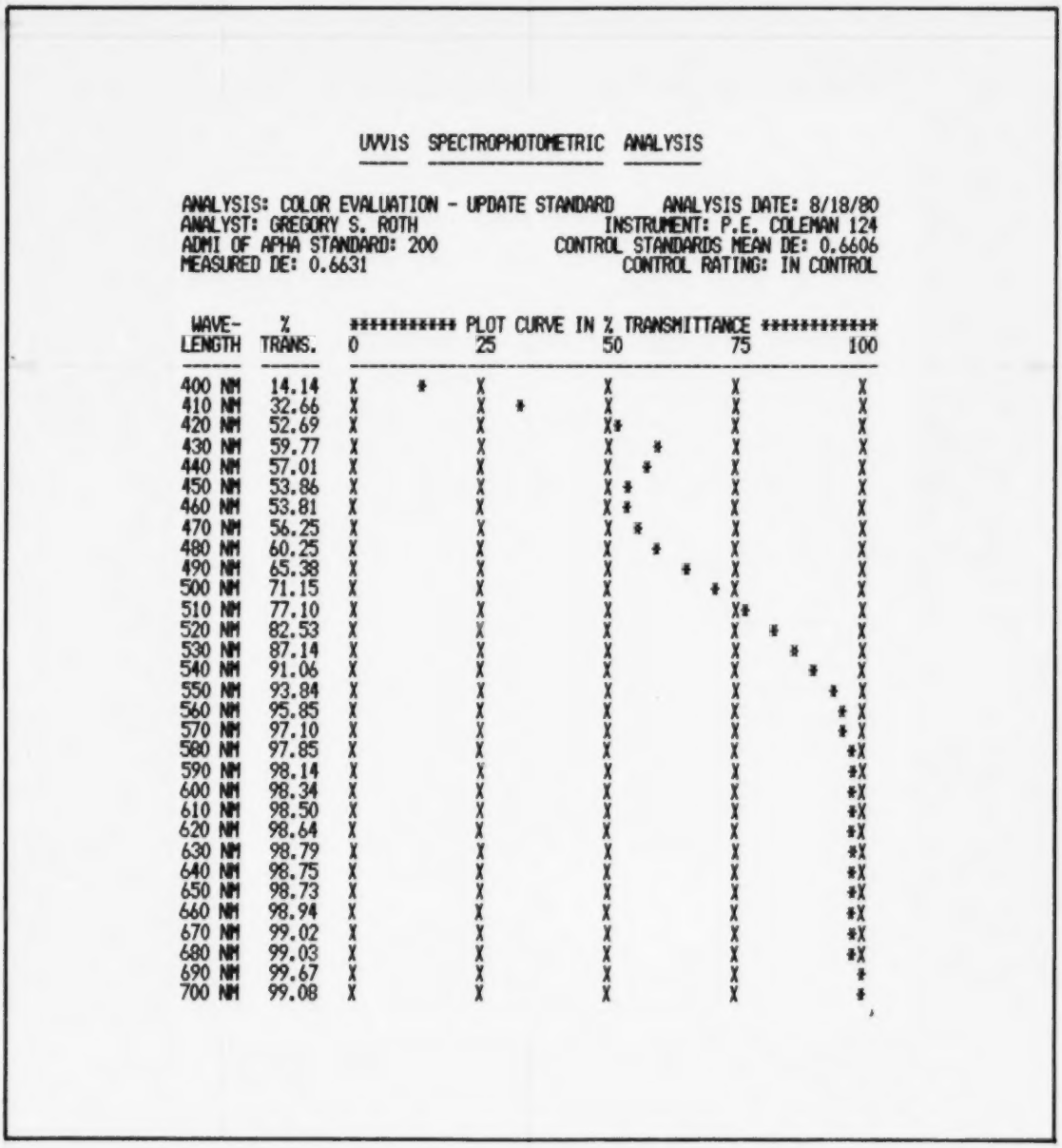


Figure 23. An example raw data plot with a grid overlay.



UVVIS SPECTROPHOTOMETRIC ANALYSIS

ANALYSIS: COLOR EVALUATION - UPDATE STANDARD ANALYSIS DATE: 8/18/80
ANALYST: GREGORY S. ROTH INSTRUMENT: P.E. COLEMAN 124
ADMI OF APHA STANDARD: 200 CONTROL STANDARDS MEAN DE: 0.6606
MEASURED DE: 0.6631 CONTROL RATING: IN CONTROL

WAVE-LENGTH	% TRANS.	********** PLOT CURVE IN % TRANSMITTANCE ********** 0 25 50 75 100
400 NM	14.14	
410 NM	32.66	
420 NM	52.69	
430 NM	59.77	
440 NM	57.01	
450 NM	53.86	
460 NM	53.81	
470 NM	56.25	
480 NM	60.25	
490 NM	65.38	
500 NM	71.15	
510 NM	77.10	
520 NM	82.53	
530 NM	87.14	
540 NM	91.06	
550 NM	93.84	
560 NM	95.85	
570 NM	97.10	
580 NM	97.85	
590 NM	98.14	
600 NM	98.34	
610 NM	98.50	
620 NM	98.64	
630 NM	98.79	
640 NM	98.75	
650 NM	98.73	
660 NM	98.94	
670 NM	99.02	
680 NM	99.03	
690 NM	99.67	
700 NM	99.08	

Figure 24. An example listed plot curve (in ten-nanometer increments).

SECTION 9

NOTES TO THE SYSTEM MANAGER

The UVVIS system consists of 19 cooperating programs and 19 data files. Eighteen of the data files have corresponding dump-file system utility programs which send file contents to the terminal and/or lineprinter. (See Table 8.)

The system is designed to have two user IDs within a shared directory. One ID permits the user to access the entire UVVIS system. The other ID allows access to only the UVVIS plot function.

The listing in the BASIC.ID file for the first ID should route control to some message-of-the-day program. This program must contain the statement:

```
CHAIN "INITIALIZE"
```

INITIALIZE is a program, resident within the UVVIS directory, which contains the statement:

```
CHAIN "UVVO.OO"
```

where UVVO.OO is the master control module of the UVVIS system.

For the second (plot-only) ID, the listing in BASIC.ID should transfer control to some message-of-the-day program which contains the statement:

```
CHAIN "INITIALIZE.2"
```

INITIALIZE.2 is a UVVIS directory-resident program which contains the statement:

```
CHAIN "UVVO.PO"
```

where UVVO.PO is the plot program of the UVVIS system.

Before the UVVIS system can be properly used, three programs must be run. These programs, which establish certain essential data files, are shown in Table 8: the 'SET' programs. The program SETOOTR will ask you to enter the following parameters:

- analyst's name (optional)
- instrument type (optional)
- transmittance signal channel number (mandatory)
- wavelength signal channel number (mandatory)
- CALL 7 scan-down channel-indicator number (mandatory)

The UVVIS directory must be INITed from the Nova background and never RELEASEd. This prevents the UNKNOWN DIR SPECIFIER error from occuring when the user of one UVVIS ID logs out while the user of the other ID is still on the system.

The filters used in the spectrophotometer quality assurance analysis may have slightly different National Bureau of Standards certified values than those presently specified in the file UVVOQ.VF. To change these values, simply alter the entries in the DATA statements within the program SETOQVF. For a linearity filter, the form of the statement will be:

```
XXXX DATA "ZZZZZ",V1,V2,V3,V4,V5
```

where XXXX is the program statement number, "ZZZZZ" is the filter designation (for example, "1-232", not to exceed five characters), and V1 through V5 are the percent transmittance values at the specified wavelengths, in order of ascending wavelength. For a tristimulus filter, the form will be:

```
XXXX DATA "ZZZZZ",V1,V2,V3,-9999,0
```

where XXXX and "ZZZZZ" are as defined above, V1 through V3 are the tristimulus values X, Y, and Z, in that order, and -9999 and 0 are integer constants. Before running SETOQVF again, make sure you execute the statement:

```
CHATR "UVVOQ.VF",""
```

to remove the permanent attribute of that file.

Should the user need to restart a run after having ended it, so as to not write over the data already accumulated, you will have to run one of the 'RESET' programs, shown in Table 8. See the table note to determine which one to run.

TABLE 8. UVVIS SYSTEM UTILITIES

Date file name	Dump-file* program	Set-file** program	Reset-file# program
UVVOO.TR	DUMPOOTR	SETOOTR	
UVVOO.CF	DUMPOOCF		
UVVOO.SF	DUMPOOSF	SETOOSF	
UVVOQ.VF	DUMPOQVF	SETOQVF	
UVVOQ.SF	DUMPOQSF		
UVVOQ.CF	DUMPOXCF		
UVVOQ.DS	DUMPOQDS		RESETOQDS
UVVOC.RP	DUMPOCRP		RESETOCRP
UVVOC.CF	DUMPOXCF		
UVVOC.DS	DUMPOCDS		
UVVOC.PR	DUMPOXPR		
UVVOC.PF	DUMPOXPF		
UVVOL.SF	DUMPOLSX		
UVVOL.SM	DUMPOLSX		
UVVOL.CF	DUMPOXCF		
UVVOL.DS	DUMPOLDS		RESETOLDS
UVVOL.PR	DUMPOXPR		
UVVOL.PF	DUMPOXPF		

* Dump-file programs dump file contents to terminal and/or lineprinter.
** Set-file programs establish the files necessary for system execution.
\# RESETOQDS for spectrophotometer quality assurance analysis; RESETOCRP for chlorophyll analysis; RESETOLDS for color analysis.

REFERENCES

1. Catalogue of NBS Standards Reference Materials. National Bureau of Standards Special Publication 260. U.S. Department of Commerce, Washington, D.C., 1975, p. 55.

2. Weber, C. I., ed. Biological Field and Laboratory Methods for Measuring the Quality of Surface Waters and Effluents. EPA-670/4-3-001, U.S. Environmental Protection Agency, Cincinnati, Ohio, 1973.

3. Allen, W., et al. Determination of Color of Water and Wastewater by Means of ADMI Color Values. In: Proceedings of the 28th Industrial Waste Conference, Purdue University, Lafayette, Indiana, 1973, pp. 661-675.

4. Office of Research and Development, Environmental Monitoring and Support Laboratory. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-79-020, U.S. Environmental Protection Agency, Cincinnati, Ohio, 1979, 110.1:1-3.

APPENDIX A

WAVELENGTH CALIBRATION CHECKING/SETTING

Should you decide to check and/or set the wavelength calibration during the preparation for an automated run, you will be given the following options:

WAVELENGTH CALIB.: CHECK[1], CALIBRATE[2], CONTINUE[3(CR)] >>>

Upon completion or termination of option 1 or 2, you will again face these options. Only the default option 3 will permit you to continue with the automated run.

Under option 1, you will first be directed to "PRESS ESC KEY TO END TESTING." Only the ESCAPE key terminates this indefinitely repeating check process. For each test, you will be directed to:

SET/ENTER WAVELENGTH AND PRESS CR KEY >>>

(CR stands for "carriage return", or the RETURN key.) First set the spectrophotometer dial at some position within the bounds of 200 to 800 nanometers. Then type in the position in nanometers, as accurately as possible. (It is easiest to set the dial directly at some integer nanometer, so that the accuracy is required in setting the dial rather than in entering the value.) Finally, press the RETURN key. The computer will make its own measurement of the wavelength position (according to the present wavelength calibration curve), and will display on your terminal this response:

EXPECTED: XXX.XX MEAS'D: YYY.YY DIFF: ±Z.ZZ CONTROL RAT'G: RRRRRRRRR

where XXX.XX is the value you entered, YYY.YY is the computer-measured value, ±Z.ZZ is the difference (YYY.YY less XXX.XX), and RRRRRRRRR is the control rating of "IN CONTROL," WARNING," OR "OUT OF CTL." (The latter rating of course means "out of control.") These control ratings are based on the following conditions, where:

±Z.ZZ < .25 nm. is "in control"

.25 nm. $\leq$ ±Z.ZZ $\leq$ .50 nm. is "warning"

±Z.ZZ > .50 nm. is "out of control"

After the test's results, the computer will again ask you for another setting/entering of a wavelength position. This is the point at which you would ESCAPE back to the wavelength calibration options.

Under option 2, you must set two factors before calibrating. The first will be displayed on your terminal as:

SELECT SETTING INCREMENT: 1) 50-NM. 2) 100-NM. >>>

Enter a 1 if you want to make readings every 50 nanometers. Enter a 2 for readings every 100 nanometers. Once you have decided this, you will be asked:

SELECT NUMBER OF READINGS PER SETTING: 1, 2, OR 3 >>>

Here you decide how many times you wish to make a reading at each of the above-chosen increments.

The calibration procedure will then ensue. The computer will request each reading by displaying on your terminal, "XXX NM. >>>" where XXX is the position at which the wavelength dial is to be set. You will set the dial and press the RETURN key. The computer will take a reading and then ask you for the next one.

The process begins at 300 nanometers, stepping up by 50 or 100 nanometers, whichever you have chosen, until 800 nanometers is reached. If you request two readings per setting, a similar sequence is done, progressing from 800 down to 300 nanometers. Should you choose three readings per setting, the 300 to 800 nanometer sequence is repeated.

After all the readings have been taken, the computer will determine the calibration curve, and display the old and new equations on your terminal. Each is of the form:

WL = ±X.XXXE±PP ±Y.YYYE±QQ * SIG + ±Z.ZZZE±RR*SIG 2

where SIG means signal (i.e. computer reading), SIG 2 means signal-squared, "*" is the symbol for multiplication, and the other numbers of the form ±X.XXXE±PP mean ±X.XXX times the PPth power of ten. Also displayed will be the standard error of estimate and the percent relative standard error of estimate. The former measures deviations of the computer readings from the expected (calibration curve) values. The latter is the standard error of estimated divided by the mean of the wavelength values.

A few things should be noted in evaluating the equation. The ±X.XXXE±PP value should be somewhat near zero. If it is more than twenty units from zero, the calibration may not be adversely affected, but you might have your system manager check the calibration of the interface box. The ±Y.YYYE±QQ value should be positive and less than one. The ±Z.ZZZE±RR value should be smaller than an order of ten to the negative fifth power. Most importantly, the standard error of estimate, for a good calibration, should be less than two tenths.

Following the calibration, you will be asked whether or not you wish to reject the new calibration results. If not, they will be stored and used throughout the run. They will also be maintained for as many subsequent runs as you wish. Wavelength calibrations which are unstable over time indicate problems within the spectrophotometer's electrical system or within the interface box. Consult your system manager in this case.

APPENDIX B

TRANSMITTANCE CALIBRATION CHECKING/SETTING

The transmittance calibration checking/setting procedure begins with some instructions. These directives, displayed on your terminal, are as follows:

1. SET WAVELENGTH AT XXX NM.
2. BLOCK SAMPLE BEAM AND ADJUST FOR 0% TRANSMITTANCE.
3. REMOVE BLOCK AND ADJUST FOR 100% TRANSMITTANCE.
4. PRESS CR KEY TO CONTINUE.

XXX is the midpoint of the scan range of the particular analysis being performed. Should the 100% transmittance adjustment require much correction, your specific instrument may require a repetition of operations 2 and 3.

Upon pressing the RETURN key, you will be offered the following options:

```
TRANSMITTANCE:  CHECK[1], CALIBRATION [2], CONTINUE[3(CR)]  >>>
```

Upon termination or completion of option 1 or 2, you will again confront these options. The default option 3 returns to you the analysis commands.

With option 1, you may make any number of tests. Should no calibration have been made, you will get a "NO TRANSMITTANCE CALIBRATION DATA FILE" error message. Otherwise, the following directives will be displayed on your terminal:

```
FOR EACH TRANSMITTANCE CHECKED,
SET WAVELENGTH AND PRESS CR KEY.
PRESS ESC KEY TO DISCONTINUE CHECKING.
```

Only by pressing the ESCAPE key can you return to the transmittance calibration options. Each check is cued by the terminal message:

```
SET WL, PRESS CR KEY  >>>
```

where WL is short for "wavelength." First set the wavelength dial anywhere within the range of the specific type of analysis you are performing. Upon pressing the RETURN key, the computer will determine the wavelength and the transmittance, and will display the results as:

```
MEAS'D % TRANS AT WWW.W NM  >>> ±TTT.TTT
```

where WWW.W is the wavelength, and ±TTT.TTT the percent transmittance. The request for another setting is displayed, at which point you may continue or press the ESCAPE key.

Note, in the checking procedure, that when you are near certain points of sudden fluctuation in the 100% transmittance line, accented by the effect of scanning past those points, the transmittance values may deviate from 100% by as much as three percent units. Consistently low readings across the entire scan range warrant a recalibration. Consistently high readings clue you to either let the instrument warm up a bit longer, or to recalibrate.

The transmittance calibration procedure begins with the following directives:

```
FOR 100% TRANSMITTANCE SCAN:
        1.  MAKE SURE BEAM BLOCK REMOVED.
        2.  SET WAVELENGTH AT XXX NM.
        3.  SET SPECTRO. FOR SCAN AND PRESS CR KEY.
```

XXX is five nanometers above the highest wavelength of the analysis' scan range. Upon setting the spectrophotometer into scanning mode, and pressing the RETURN key, the scan and data collection begins. Should you receive the following error message:

```
PROBLEM IN SCAN RANGE; SET WAVELENGTH AT XXX NN.,
```

then turn off the scanning mode, set the wavelength to the position XXX nanometers, turn of the scanning mode, and press the RETURN key to continue the scan. Whenever this error keeps recurring, then ESCAPE back to the analysis commands, and check the scanning mechanism speed (which is an option under the M command).

Once the 100% transmittance scan has been made, the following instructions will be displayed on your terminal:

```
FOR 0% TRANSMITTANCE SCAN:
      1.  INSERT BEAM BLOCK.
      2.  SET WAVELENGTH AT XXX NM.
      3.  SET SPECTRO. FOR SCAN AND PRESS CR KEY.
```

XXX is as defined immediately above. The second scan procedure is initiated when you press the RETURN key.

With the fulfillment of the zero percent transmittance scan, the computer will inform you of the calibrations's completion, and will ask:

```
IGNORE NEW CALIBRATION DATA ?  [N(CR) OR Y]  >>>
```

Enter an N (or simply press the RETURN key) to save the new calibration. Only a Y answer will direct the computer to ignore the new data.

APPENDIX C

SCANNING MECHANISM SPEED CHECKING/SETTING

When checking and/or setting the scanning mechanism speed, you are given the following options:

SCAN MECH. SPEED: CHECK[1], SET[2], OR CONTINUE[3(CR)] >>>

Completion of option 1 or 2 will return to you these options. Only the default option 3 will again give you the analysis commands.

Under option 1, you are first given the following directions:

SCANNING MECHANISM SPEED CHECK:
1. SET WAVELENGTH AT SCANNING MID-RANGE.
2. SET SPECTRO. FOR SCAN OPERATION AND PRESS CR KEY.

Upon completing these tasks and pressing the RETURN key, a five second scan will be made. Results are then displayed on your terminal as:

EXPECTED SPEED: XXX NM/MIN MEAS'D SPEED: YYY NM/MIN DIFF: ±ZZZ NM/MIN

where XXX is the presently set speed, YYY is the speed just measured, and ±ZZZ is the difference (YYY less XXX).

Under option 2, instructions and operations like those of option 1 initiate a ten second scan. (The only difference is the first instruction line: "SCANNING MECHANISM SPEED SET.") The scan results are shown on your terminal as:

MEASURED SCAN SPEED IS APPROX. XXX NM/MIN

IGNORE NEWLY SET SCAN SPEED ? [N(CR) OR Y] >>>

XXX is the measured speed to the nearest five nanometers per minute. If you accept the results by entering an N (or simply pressing the RETURN key), then the new speed will be stored. (It might be wise to verify it with the check option.) Only the Y answer permits the new speed to be ignored.

You may get an error message of the form:

MEASURED SPEED VARIED FROM XXX.XX NM/MIN TO YYY.YY NM/MIN

Where XXX.XX and YYY.YY are the variant speeds. This message accompanies an overly fluctuating measured scan speed. This sometimes happens just after switching from one speed to another. It is suggested that you try the check or setting operation again.

APPENDIX D

OBTAINING REPORTS

Whenever a report has been fully prepared by the system, you are given three options which are stated in the following form:

```
XXXXXXXXXX  TERMINAL[1], LINEPRINTER[2], CONTINUE[3(CR)]  >>>
```

where XXXXXXXXXX is some report description, such as "SAMPLE REPORT."

Option 1 sends the report to your terminal. These reports are free of page headings other than that of the front page. Once the entire report has been displayed, you are again given the three options.

Option 2 sends the report to the lineprinter associated with your Nova minicomputer. These reports may sometimes have more than one page. On occasion, someone else may be sending a report to the lineprinter. In this case, you should note the accompanying terminal message:

```
LINEPRINTER IN USE; WILL DELAY 10 SECS. AND RETRY.
```

Should this message continually recur, consult you system manager. Once the report has been completely transmitted to the lineprinter, the three options will again be offered to you.

Option 3, the default option, returns you to the particular command line from which you chose the report option.